Bioaerosols: Assessment and Control

Editor:

Janet Macher, Sc.D., M.P.H.

Assistant Editors:

Harriet A. Anunann, Ph.D., D.A.B.T.
Harriet A. Burge, Ph.D.
Donald K. Milton, M.D., Dr.P.H.,M.O.H.
Philip R. Morey, Ph.D., C.I.H.

ACGIH
1330 Kemper Meadow Drive
Cincinnati, OH 45240-1634
www.acgih.org

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ACGIH
Kemper Woods Center
1330 Kemper Meadow Drive
Cincinnati, OH 45240-1634
Telephone: 513-742-6163 Fax: 513-742-3355
E-mail: comm@acgih.org
http://www.acgih.org

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FOREWORD

When Theodore Hatch reviewed the advances made in preventing occupational disease in the first half of this century, he observed that progress had been made as a result of interdisciplinary collaboration "... a true melding, even to the point where the different individuals making up the group lost sight of their respective fields and functioned together as a unit, each making his own peculiar contribution, but always as part of the whole" (Hatch, 1964). This book, produced by the ACGIH Bioaerosols Committee in 1998, is a perfect example of the continued progress we can make by following Hatch's guidance. In this case, the product is a comprehensive guide to the assessment and control of bioaerosols. A glance at the list of editors and chapter authors reveals the remarkable diversity in professional backgrounds and specialties of the contributors. This group has converged upon the need to prevent occupational disease by applying the industrial hygiene paradigm of recognition, evaluation, and control to the set of hazards loosely termed *bioaerosols*. The result is *Bioaerosols: Assessment and Control*, which will help the industrial hygienist, indoor environmental specialist, and other occupational health professionals learn from the wide range of people who contributed their expertise to advance the state of knowledge of biologically derived airborne contaminants. Hatch would have been proud.

There is a small measure of irony in the publication of this book by an organization in a profession which still identifies itself by title as "industrial." The ACGIH Bioaerosols Committee has prepared guidance which will be useful in the full range of workplaces, including those which do not fit the traditional image of "industrial." These indoor workplaces, are in fact the environments in which most people spend their working time. The ubiquitous nature of biologically derived contaminants, and their importance as causal agents of work-related disease, are actually among the factors which forced our profession to broaden its scope to the non-industrial workplace. As workers in these apparently benign environments began to demonstrate symptoms which they attributed to their workplace, industrial hygienists first applied the tools we had available for evaluating the manufacturing environment. In many cases, the measurements of chemicals as gases, vapors, and aerosols revealed exposures far below the levels we had found in the manufacturing world. There was a tendency to dismiss the workers' complaints, because the exposures we measured in their environments did not approach the levels we had become accustomed to finding in the workplaces where industrial hygienists had traditionally practiced. In retrospect, it is clear that we were wrong to dismiss the workers for at least two reasons. First, we tended to discount evidence of a causal association between symptoms and the workplace because the exposure levels were below the limits. This was done despite the admonitions of the TLV Committee and others that the exposure limits are not meant to be interpreted as hard lines between safe and unsafe conditions. Second, we were not sufficiently aware of the limitations of our measurement methods, especially the fact that we usually made no measurements at all of the biologically derived contaminants which are the subject of this book. As the ACGIH guide clearly documents, an investigation of work-related disease must include some assessment of possible exposure to biologically derived contaminants, and this book will put this important tool into the hands of future investigators.

Finally, the book illustrates the application of the scientific method to the study of occupational hazards and disease. This is remarkable only by comparison to the conventional approach to industrial hygiene, which is frequently compliance-driven. But how does one design and conduct an investigation in a setting where there are no relevant exposure limits? The approach the Bioaerosols Committee has presented in this book is founded upon the scientific method of investigating potential causal relationships between exposure and disease. It is a thoughtful application of the scientific approach to workplace investigations which can and should be used over the full range of exposures and hazards, not just the biologically derived contaminants which are the subject of this book.

So *Bioaerosols: Assessment and Control* presents in a single volume a comprehensive guide to the recognition, evaluation, and control of biologically derived contaminants. It is a remarkable product of volunteer effort, and it represents a significant step toward the improvement of workplace conditions.

Robert F. Herrick, Sc.D., C.I.H.

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CONTRIBUTORS

Harriet M. Ammann, Ph.D., D.A.B.T.
Office of Environmental Health
Assessment Services
Department of Health
Olympia, Washington

Larry G. Arlian, Ph.D.
Department of Biological Sciences
Wright State University
Dayton, Ohio

Harriet A. Burge, Ph.D.
Department of Environmental Health
Harvard School of Public Health
Boston, Massachusetts

Eugene C. Cole, Dr.P.H.
Dyncorp
Durham, North Carolina

Karin K. Foarde, M.S.
Research Triangle Institute
Research Triangle Park,
North Carolinia

Kathleen Kreiss, M.D.
NIOSH
Morgantown, West Virginia

Janet M. Macher, Sc.D., M.P.H.
Environmental Health Laboratory
California Department of Health
Services
Berkeley, California

Kenneth F. Martinez, M.S.E.E., C.I.H.
NIOSH
Cincinnati, Ohio

John W. Martyny, Ph.D., C.I.H.
Tri-County Health Department
Commerce City, Colorado

Donald K. Milton, M.D., Dr.P.H., M.O.H.
Occupational Health Program
Harvard School of Public Health
Boston, Massachusetts

Philip R. Morey, Ph.D., C.I.H.
Microbiology, AQS Services
Gettysburg, Pennsylvania

Edward A. Nardell, M.D.
The Cambridge Hospital
Cambridge, Massachusetts

James A. Otten, S.M.
Camber Corporation
Oak Ridge, Tennesee

Cecile S. Rose, M.D., M.P.H.
Occupational and Pulmonary Medicine
National Jewish Medical and
Research Center
Denver, Colorado

Richard Shaughnessy, Ph.D.
University of Tulsa, CERT
Tulsa, Oklahoma

Klaus Willeke, Ph.D., C.I.H.
Department of Environmental Health
University of Cincinnati
Cincinnati, Ohio

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Bioaerosols Committee Members

Harriet M. Ammann, Ph.D., D.A.B.T.
Harriet A. Burge, Ph.D.
Kathleen Kreiss, M.D. (retired)
Janet M. Macher, Sc.D., M.P.H.
Kenneth F. Martinez, M.S.E.E., C.I.H.
John W. Martyny, Ph.D., C.I.H.
Donald K. Milton, M.D., Dr.P.H.
Philip R. Morey, Ph.D., C.I.H.
Edward A. Nardell, M.D.
James A. Otten, S.M.
Tiina Reponen, Ph.D. (new appointment)
Stephen J. Reynolds, Ph.D., C.I.H. (new appointment)
Cecile S. Rose, M.D., M.P.H. (retired)
Richard Shaughnessy, Ph.D.
Klaus Willeke, Ph.D., C.I.H. (retired)

ABBREVIATIONS/ACRONYMS/UNITS/LATIN NAMES

AGENCIES AND ASSOCIATIONS

AAAAI	American Academy of Allergy, Asthma, and Immunology
AATCC	American Association of Textile Chemists and Colorists
ACGIH	American Conference of Governmental Industrial Hygienists
AIHA	American Industrial Hygiene Association
AOAC	Association of Official Analytical Chemists
ARC	American Red Cross
ASHRAE	American Society of Heating, Refrigerating and Air-Conditioning Engineers
ASTM	American Society for Testing and Materials
CDC	Centers for Disease Control and Prevention
CEN	European Standardization Committee
CMHC	Canada Mortgage and Housing Corporation
FEMA	Federal Emergency Management Agency
IAACI	International Association of Allergology and Clinical Immunology
ICRP	International Commission on Radiological Protection
IICRC	Institute of Inspection, Cleaning and Restoration Certification
IOM	Institute of Medicine
ISIAQ	International Society of Indoor Air Quality and Climate
ISO	International Standards Organization
IUATLD	International Union Against Tuberculosis and Lung Disease
NADCA	National Air Duct Cleaners Association
NAIN	National Antimicrobial Information Network
NIOSH	National Institute for Occupational Safety and Health
NIST	National Institute of Standards and Technology
USEPA	U.S. Environmental Protection Agency
USP	U.S. Pharmacopeia

ABBREVIATED AGENT NAMES — Amebae

N. fowleri	*Naegleria fowleri*

ABBREVIATED AGENT NAMES — Arthropods and Arthropodic Antigens

A. siro	*Acarus siro* (storage mite)
B. tropicalis	*Blomia tropicalis* (house dust mite)
Bla g	*Blattella germanica* (German cockroach) allergen
D. farinae	*Dermatophagoides farinae* (house dust mite)
Der f	*Dermatophagoides farinae* (allergen)
D. microceras	*Dermatophagoides microceras* (house dust mite)
D. pteronyssinus	*Dermatophagoides pteronyssinus* (house dust mite)
Der p	*Dermatophagoides pteronyssinus* (allergen)
E. maynei	*Euroglyphus maynei* (house dust mite)
L. destructor	*Lepidoglyphus destructor* (storage mite)
Per a	*Periplaneta americana* (American cockroach) (allergen)

ABBREVIATED AGENT NAMES — Bacteria

E. coli	*Escherichia coli*
L. bozemanii	*Legionella bozemanii*
L. jordanis	*Legionella jordanis*
L. micdadei	*Legionella micdadei*
L. pneumophila	*Legionella pneumophila*
M. tuberculosis	*Mycobacterium tuberculosis*
S. aureus	*Staphylococcus aureus*
S. pneumoniae	*Streptococcus pneumoniae*

ABBREVIATED AGENT NAMES — Fungi

A. obclavatum	*Acremonium obclavatum*
A. flavus	*Aspergillus flavus*
A. fumigatus	*Aspergillus fumigatus*
A. niger	*Aspergillus niger*
A. parasiticus	*Aspergillus parasiticus*
A. versicolor	*Aspergillus versicolor*
B. dermatitidis	*Blastomyces dermatitidis*
B. cineria	*Botrytis cineria*
C. albicans	*Candida albicans*
C. herbarum	*Cladosporium herbarum*
C. sphaerospermum	*Cladosporium sphaerospermum*
C. immitis	*Coccidioides immitis*
C. sativus	*Cochliobolus sativus*
C. neoformans	*Cryptococcus neoformans*
E. nidulans	*Emericella nidulans*
E. nigrum	*Epicoccum nigrum*
F. moniliforme	*Fusarium moniliforme*
H. capsulatum	*Histoplasma capsulatum*
P. aurantiogriseum	*Penicillium aurantiogriseum*
P. chrysogenum	*Penicillium chrysogenum*
P. commune	*Penicillium commune*
P. fastigata	*Phialophora fastigata*
P. roqueforti	*Penicillium roqueforti*
S. atra	*Stachybotrys atra*
S. chartarum	*Stachybotrys chartarum*

ABBREVIATED AGENT NAMES — Mammals and Mammalian Antigens

Can f	*Canis familiaris* (domestic dog) allergen
Fel d	*Felis domesticus* (domestic cat) allergen

ABBREVIATED AGENT NAMES — Viruses

FCV	Four Corners virus
HIV	human immunodeficiency virus
SNV	Sin Nombre virus

CHEMICALS AND CHEMICAL FORMULAS

$Ca(OCl)_2$	calcium hypochlorite
Cl	chlorine
ClO_2	chlorine dioxide
CO_2	carbon dioxide

DAPI	4',6-diamidino-2-phenyl-indole
ETO	ethylene oxide
FITC	fluorescein isothiocyanate
FRC	free residual chlorine
H_2O	water
H_2O_2	hydrogen peroxide
HCHO	formaldehyde
K_2HPO_4	dipotassium hydrogen phosphate
$MgSO_4$ 7 H_2O	magnesium sulfate heptahydrate
NaOCl	sodium hypochlorite
O_3	ozone

CONDITION AND DISEASE NAMES

ABPA	allergic bronchopulmonary aspergillosis
ABPM	allergic bronchopulmonary mycosis
AIDS	acquired immunodeficiency syndrome
ATA	alimentary toxic aleukia
BRI	building-related illness
BRS	building-related symptom
GAE	granulomatous amebic encephalitis
HP	hypersensitivity pneumonitis
HPS	Hantavirus pulmonary syndrome
MCS	multiple-chemical sensitivity
MDR-TB	multiple-drug resistant tuberculosis
NSBRS	non-specific building-related symptom
ODTS	organic dust toxic syndrome
PAM	primary amebic meningoencephalitis
RADS	reactive airway disease syndrome
RUDS	reactive upper airway disease syndrome
SBS	sick building syndrome
SBRI	specific building-related illness
TB	tuberculosis

OTHER ABBREVIATIONS IN TEXT

AGI	all-glass impinger
AND-I	Andersen impactor, stage 1
AND-VI	Andersen impactor, stage 6
AO	acridine orange
BASE	Building Assessment Survey and Evaluation
BCE	before current era
BRI	building-related illness
BRS	building-related symptom
BURK	Burkard spore trap
CA	cellulose acetate
CBF	ciliary beat frequency
CE	cellulose mixed ester
CFR	Code of Federal Regulations
CFU	colony-forming unit
CNS	central nervous system
CT	computerized tomography
CT	cooling tower
CV	coefficient of variation
DD	dilution-dependent inhibition
DG	dichloran glycerol

DII	dilution-independent inhibition
DNA	deoxyribonucleic acid
EC	evaporative cooler
EHP	environmental health professional
ELISA	enzyme-linked immunosorbent assay
EMPAT	Environmental Microbiology Proficiency Analytical Testing
ERH	equilibrium relative humidity
ESP	electrostatic precipitator
FID	flame ionization detector
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GC	gas chromatography
GC-MS	gas chromatography-mass spectrometry
GF	glass fiber
GNB	Gram-negative bacterium
GPB	Gram-positive bacterium
Gr.	Greek
HEPA	high-efficiency particulate air
HPLC	high performance liquid chromatography
HVAC	heating, ventilating, and air conditioning
IEQ	indoor environmental quality
IgA	immunoglobulin A
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
IH	industrial hygienist
LAL	*Limulus* amebocyte lysate
LC	liquid chromatography
LDL	lower detection limit
LPS	lipopolysaccharide
LTA	lipoteichoic acid
MC	moisture content
MEA	malt extract agar
MK-II	New Brunswick Slit Sampler
MS	mass spectrometry
MS-MS	dual or tandem mass spectrometry
MSDS	material safety data sheet
MVOC	microbial volatile organic compound
MWF	metal-working fluid
NSBRS	Non-specific building-related symptoms
OAI	outdoor air intake
PC	polycarbonate
PCR	polymerase chain reaction
PG	peptidoglycan
pH	hydrogen-ion activity
PID	photoionization detector
PM10	particulate matter <10 m
PMN	polymorphonuclear neutrophil
PPE	personal protective equipment
PVC	polyvinyl chloride
R	agreement ratio
RAST	radio-allergosorbent test
RCS	Reuter centrifugal sampler
RH	relative humidity
RLV	relative limit value
RNA	ribonucleic acid

SAS	surface air system
SBS	sick building syndrome
SBRI	Specific building-related illness
SENSOR	Sentinel Event Notification System for Occupational Risks
sp.	species (singular)
spp.	species (plural)
TLC	thin layer chromatography
TLV	Threshold Limit Value
TST	tuberculin skin test
UDL	upper detection limit
UV	ultraviolet
UVGI	ultraviolet germicidal irradiation
UVR	ultraviolet radiation
VOC	volatile organic compound
X	times (magnification)

UNITS OF MEASURE

µg	microgram (10^{-6} gram)
µg/g	microgram per gram
µg/m²	microgram per square meter
µg/m³	microgram per cubic meter
µg/mL	microgram per milliliter
µm	micrometer (10^{-6} meter)
°C	degree Celsius
°F	degree Fahrenheit
ACH	air change per hour
atm	atmosphere
a_w A_w	water activity
CFU	colony-forming unit
CFU/cm²	colony-forming unit per square centimeter
CFU/g	colony-forming unit per gram
CFU/m³	colony-forming unit per cubic meter
CFU/mg	colony-forming unit per milligram
CFU/mL	colony-forming unit per milliliter
cm	centimeter (10^{-2} meter)
cm/s	centimeter per second
EU	endotoxin unit
EU/g	endotoxin unit per gram
EU/m³	endotoxin unit per cubic meter
FEV_1	forced expiratory volume in one minute
ft	foot
ft²/gal	square foot per gallon
ft³/min	cubic foot per minute
g	gram
g/cm³	gram per cubic centimeter
g/L	gram per liter
g/m²	gram per square meter
g/m³	gram per cubic meter
gal	gallon
in	inch
in/ft	inch per foot
in wg	inch water gage
kDa	kilodalton (10^3 dalton)
kg	kilogram (10^3 gram)
kg/m³	kilogram per cubic meter

L	liter
L/min	liter per minute
L/s	liter per second
lb	pound
lb/ft^3	pound per cubic foot
m	meter
m/s	meter per second
m^3/day	cubic meter per day
m^3/hour	cubic meter per hour
m^3/min	cubic meter per minute
mg	milligram (10^{-3} gram)
mg/g	milligram per gram
mg/kg	milligram per kilogram
mg/L	milligram per liter
mg/m^3	milligram per cubic meter
mil	0.001 inch
min	minute
mL	milliliter (10^{-3} liter)
mm	millimeter (10^{-3} meter)
ng	nanogram (10^{-9} gram)
ng/g	nanogram per gram
ng/m^3	nanogram per cubic meter
ng/mg	nanogram per milligram
nm	nanometer (10^{-9} meter)
Pa	Pascal
pg	picogram (10^{-12} gram)
pg/m^3	picogram per cubic meter
pH	hydrogen-ion activity
ppm	part per million
RH	relative humidity
s	second

VARIABLES IN TEXT AND EQUATIONS

α	significance level
Δ	change
δ	surface density
η	fluid viscosity
ρ_p	particle density
Σ	summation
τ	particle relaxation time
χ^2	chi-square
A	deposit area
A	total number of species in Sample 1
B	total number of species in Sample 2
C	number of cases
c_a	average air concentration
C_c	Cunningham correction factor
d	particle diameter
d_a	aerodynamic particle diameter
d_{50}	50% cut-off diameter
d_i	rank difference in Spearman rank correlation
E_c	cumulative error
E_i	individual error
f	degrees of freedom

GM	geometric mean
GSD	geometric standard deviation
H	test statistic in Kruskal-Wallis procedure
H_0	null hypothesis
H_1	alternative hypothesis
I	number of infectious persons
ID_{50}	50% infectious dose
k	number of columns in two-way analysis of variance
k	number of populations in Kruskal-Wallis procedure
L	length, slit impactor
LD_{50}	50% lethal dose
m	number of items in first sample in two-sample Wilcoxon test
n	sample size
n	number of items in second sample in two-sample Wilcoxon test
n	number of isolates in Spearman rank correlation
n	number of rows in two-way analysis of variance
n_i	number of items in each sample in Kruskal-Wallis procedure
N	sum of n_i in Kruskal-Wallis procedure
p	breathing rate
P	significance level
q	generation rate of infectious agents
Q	airflow rate, ventilation rate
R	agreement ratio
R_i	mean ranks in Kruskal-Wallis procedure
R_j	sum of ranks in two-way analysis of variance
r_s	Spearman rank correlation
S	number of susceptible persons
S	particle stopping distance
S_{50}	50% stopping distance
SD	standard deviation
Stk	Stokes number
Stk_{50}	Stokes number 50% of particles
t	time
T	correction for ties in Kruskal-Wallis procedure
U_o	initial particle velocity
W	width (impactor nozzle)
W	number of species two samples have in common
x_i	individual measurement
$\bar{x}$	sample mean
Y_0	zone height above impaction surface within which incoming streamlines are deflected from original paths
Z	impactor jet-to-plate distance
Z_c	greatest distance from an impaction plate within which air moves laterally

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Chapter 1

Introduction

1.1 Bioaerosols and Biologically Derived Airborne Contaminants

Bioaerosols are those airborne particles that are living or originate from living organisms. Bioaerosols include microorganisms (i.e., culturable, nonculturable, and dead microorganisms) and fragments, toxins, and particulate waste products from all varieties of living things. Bioaerosols are ubiquitous in nature and may be modified by human activities. All persons are repeatedly exposed, day after day, to a wide variety of such materials. Individual bioaerosols range in size from submicroscopic particles (<0.01 μm) to particles greater than 100 μm in diameter.

ACGIH uses the term *biologically derived airborne contaminants* to describe bioaerosols, gases, and vapors that living organisms produce. Biologically derived materials are natural components of indoor and outdoor environments but, under some circumstances, biological agents may be considered contaminants when found indoors. ACGIH has defined *biological contamination* in buildings as the presence of (a) biologically derived aerosols, gases, and vapors of a kind and concentration likely to cause disease or predispose persons to adverse health effects, (b) inappropriate concentrations of outdoor bioaerosols, especially in buildings designed to prevent their entry, or (c) indoor biological growth and remnants of growth that may become airborne and to which people may be exposed (ACGIH, 1998a). The term *biological agent* is used here to refer to a substance of biological origin that is capable of producing an effect, for example, an infection, a hypersensitivity, irritant, inflammatory, or other response.

1.2 Why No TLVs® for Bioaerosols

Threshold Limit Values (TLVs) refer to air concentrations of substances and represent conditions under which it is believed that nearly all workers may be repeatedly exposed day after day without adverse health effects (ACGIH, 1998b). Standards to prevent harmful exposures to air contaminants have five primary components: (a) the criterion or scientific basis for the standard, (b) a sampling method, (c) an analytical method, (d) a sampling strategy, and (e) a limit value. ACGIH has considered the possibility of recommending TLVs for bioaerosols and concluded that sufficient information is not yet available on these five components for many of the biologically derived airborne contaminants to which workers are exposed in non-manufacturing environments (ACGIH, 1998a). The statement on why there are so few TLVs for bioaerosols is repeated and expanded here.

TLVs exist for certain substances of biological origin, including cellulose; some wood, cotton, and grain dusts; nicotine; pyrethrum; starch; subtilisins (proteolytic enzymes); sucrose; vegetable oil mist; and volatile compounds produced by living organisms (e.g., ammonia, carbon dioxide, ethanol, and hydrogen sulfide). However, there are no mandatory numerical limits against which investigators can compare measurements of air or source concentrations for the majority of substances of biological origin that are associated with building-related exposures. Thus, in the U.S., sampling for biologically derived airborne contaminants is not conducted for the purpose of complying with any federal or state regulations other than for the agents for which existing TLVs have been adopted as standards. Readers should be aware of any national or local standards for exposure to biological agents that have been set elsewhere than in the U.S. Where such standards exist, investigators should use appropriate procedures to measure exposures to specific biological agents and follow applicable guidelines for interpreting exposure measurements.

Given the general absence of compliance monitoring for biological agents, data on the range of inhalation exposures to specific biological agents are limited and the methods that investigators use to collect and analyze these agents vary widely. Even if limits were set, at present they would be arbitrary standards because the available environmental and health data on which to base exposure criteria are few and of inconsistent quality, as described in more detail below. Further, there are more reliable methods to identify environments in need of intervention than by comparing environmental bioaerosol measurements with numerical standards. This book (developed by a consensus of researchers and practitioners under the auspices of ACGIH) defines methods for assessing and controlling exposures to biologically derived airborne contaminants. These methods rely on visually inspecting buildings, assessing occupant symptoms, evaluating building performance, testing potential environmental sources, and applying professional judgement.

1.2.1 Sampling for Airborne Biological Agents

This book provides background information on the major groups of bioaerosols including their sources and health effects. Also described here are methods to collect, analyze, and interpret samples for biological agents from potential environmental sources. Occasionally, environmental sampling detects a single or predominating biological agent. More commonly, environmental sampling reveals a mixture of many biologically derived materials, reflecting the diverse and interactive nature of indoor microenvironments. Some of these biological agents are clearly harmful and their confirmed presence is a cause for concern. Other biological materials are normal components of indoor and outdoor environments. These latter agents typically cause little, if any, harm but may elicit responses in sensitive persons, even at ambient concentrations, or in all persons, when such agents are present in sufficient quantity. Environmental sampling for bioaerosols should only be conducted following the careful formulation of testable hypotheses about potential sources of biological agents and mechanisms by which workers may be exposed to bioaerosols from these sources. Even when investigators work from testable hypotheses and well-formulated sampling plans, results from environmental bioaerosol studies may be inconclusive and occasionally even misleading.

For the reasons identified below, there are no TLVs for interpreting environmental measurements of (a) total culturable or countable bioaerosols (e.g., total bacteria or fungi), (b) specific culturable or countable bioaerosols (e.g., *Aspergillus fumigatus*), (c) infectious agents (e.g., *Legionella pneumophila*), or (d) assayable biological contaminants (e.g., endotoxin, mycotoxins, allergens, or microbial volatile compounds).

1.2.2 Total Culturable or Countable Biological Agents

Culturable biological agents are those bacteria and fungi that can be grown in laboratory culture. The results of such cultures are reported as the number of colony-forming units per sample volume, mass, or area. Countable bioaerosols are those pollen grains, fungal spores, bacterial cells, and other particles that can be identified and counted under a microscope. The results of such samples are reported as the number of particles per sample volume, mass, or area. A general exposure limit for concentrations of culturable or countable biological agents is not scientifically supportable because of the following:

1. Culturable microorganisms and countable biological particles do not comprise a single entity. Bioaerosols in occupational settings are generally complex mixtures of many different microorganisms as well as non-living particles released from fungi, bacteria, animals, and plants.
2. Human responses to bioaerosols range from innocuous effects to serious, even fatal, diseases, depending on the specific material involved and workers' susceptibility to it. Therefore, an appropriate exposure limit for one bioaerosol may be entirely inappropriate for another. Exposure limits for total culturable or countable biological agents would not take these differences into consideration and, therefore, no such recommendations are given in this book.
3. It is not possible to collect and evaluate all bioaerosol components using a single sampling method. Many reliable air sampling methods are available to collect bioaerosols and many sensitive and specific analytical methods are available to assay biological agents. However, different methods of sample collection and analysis may result in different estimates of the relative concentrations of culturable or countable bioaerosols. Therefore, any exposure limits that may be proposed for total culturable or countable biological agents must specify the methods of bioaerosol collection and analysis.
4. At present, information relating culturable or countable bioaerosol concentrations to health effects is generally insufficient to describe exposure–response relationships from which TLVs could be derived.

To overcome the obstacles listed above, information would be needed on the concentrations of the diverse culturable and countable biological agents that may be found in work, residential, commercial, and recreational environments. These data would have to be compiled consistently using carefully chosen methods of sample collection and analysis. In addition to environmental measurements, matching data on health responses for the

occupants of the tested environments would have to be collected to determine if there is any value in measuring total culturable or countable biological agents to predict environmental air quality. It would also be valuable to determine where and when air sampling for total culturable or countable bioaerosols may identify environmental contamination that could not otherwise be detected by careful visual examination, questioning of facility operators and occupants, or other means of assessing the potential presence of harmful or undesirable biological agents.

1.2.3 Specific Culturable or Countable Bioaerosols Other than Infectious Agents

Exposure limits for individual culturable or countable bioaerosols have not been established to prevent hypersensitivity, irritant, or toxic responses. At present, information relating health effects with exposures to specific culturable or countable bioaerosols consists largely of case reports and qualitative exposure assessments. Future information on exposure–response relationships may lead to the establishment of exposure limits. However, currently available data are generally insufficient for this purpose. Some obstacles to collecting good epidemiological data on dose–response relationships for specific culturable or countable biological agents include the following:

1. Most data on concentrations of specific bioaerosols are derived from indicator measurements where the relationship of the indicator to the concentration and biological activity of the actual effector agent is unknown. For example, investigators often use the air concentration of culturable fungi to represent potential exposure to airborne fungal allergens and mycotoxins. The accuracy of substituting exposure to airborne fungi for exposure to fungal allergens or toxins has not been determined.
2. Replicate sampling is uncommon in bioaerosol assessments despite the fact that bioaerosol components and relative concentrations vary widely within and among different occupational and environmental settings. Further, the most commonly used air sampling devices for bioaerosol testing are designed to collect "grab" samples over relatively short time intervals. Measurements from single, short-term grab samples are poor predictors of either short-term or long-term air concentrations. Long-term average air concentrations are good predictors for some bioaerosol-related hazards, but grab samples may be orders of magnitude higher or lower than average concentrations and are unlikely to represent workplace exposures accurately. Some organisms and sources release aerosols as "concentration bursts," which may only rarely be detected by limited grab sampling and may be masked in measurements of long-term average concentrations. Nevertheless, such episodic bioaerosol releases may produce significant health effects.
3. In studies of single workplaces, the number of persons affected by exposure to biological agents may be small if contamination is localized and affects only a fraction of building occupants. However, data from different studies can seldom be combined to reach meaningful numbers of test subjects because the specific types of biological agents responsible for bioaerosol-related illnesses are diverse and often differ from study to study. These factors contribute to the low statistical power common in evaluations of cause–effect relationships between exposures to specific biological agents and building-related health complaints.

To overcome the obstacles listed above, better sampling and analytical methods for specific biological agents are needed to estimate exposures to actual effector agents. Also needed are detailed and extensive experimental or epidemiological data on the magnitudes and patterns of exposures to specific biological agents as well as careful and objective measurements of health effects.

1.2.4 Infectious Agents

Human dose–response data are available for only a few infectious bioaerosols. At present, air sampling protocols for infectious agents (other than some opportunistic pathogens) are limited and suitable primarily for research endeavors. Therefore, environmental sampling for infectious agents is not a routine approach to assessing their presence and worker exposure thereto. Rather, infectious diseases are controlled through awareness of the potential for infection and surveillance of workers at risk of exposure to infectious agents. In most routine exposure settings, public health measures (e.g., immunization, post-exposure prophylaxis, active case finding, and medical treatment) remain the primary defenses against infectious bioaerosols. In addition to these measures, facilities associated with increased risks for transmission of airborne infectious diseases (e.g., microbiology laboratories, animal handling facilities, and health-care settings) should employ work practices and engineering controls to minimize air concentrations of infectious agents. Further, such facilities should consider the need for administrative controls and, where contact cannot otherwise be avoided, the use of personal protective equipment to prevent the exposure of workers to infectious aerosols. This book also discusses the use of protective equipment in remediation

work that may involve skin or inhalation exposure to infectious agents in contaminated building materials or organic debris.

1.2.5 Assayable Biological Contaminants

Assayable, biologically derived contaminants (e.g., endotoxin, mycotoxins, allergens, and microbial volatile compounds) are fungal, bacterial, animal, or plant substances that can be detected using chemical, immunological, or biological assays. Evidence does not yet support exposure limits for any of these substances. However, collection and assay methods for certain common airborne allergens and endotoxin are steadily improving, and field validation of these methods is also progressing. Dose–response relationships for some assayable bioaerosols have been observed in experimental studies and occasionally in epidemiological surveys. Therefore, a relative exposure limit for endotoxin is proposed in this book and exposure limits for other substances may be appropriate in the future. Innovative molecular techniques are becoming available for specific bioaerosols currently detectable only by culture or counting. Use of these techniques may provide more information from which to understand exposure–response relationships for assayable biological agents.

1.2.6 Criteria on Which to Base Exposure Limits for Biologically Derived Airborne Contaminants

Many of the difficulties faced in attempts to establish TLVs for biologically derived airborne contaminants were previously encountered with other substances. Established limits for chemical substances are not fine lines between safe and dangerous concentrations nor are they a relative index of toxicity. A similar caveat would apply to TLVs that may eventually be considered for workplace exposures to biological agents. Because of wide variation in individual susceptibility to chemical and biological agents, a small percentage of workers may experience discomfort from some substances at concentrations at or below a threshold limit. A smaller percentage of workers may be affected more seriously by aggravation of a pre-existing condition or by development of an occupational illness. Among the parameters that may affect workers' reactions to substances are genetic factors, age, personal habits, medication, or previous exposures.

The bases on which TLVs are established may differ from substance to substance because of the diversity of chemical and biological agents to which workers may be exposed and the ranges of health responses workers may have as a consequence of these exposures. Protection against impairment of health may be a guiding factor for some exposure limits, whereas reasonable freedom from irritation, narcosis, nuisance, or other forms of stress may form the basis for others (ACGIH, 1998b). Health impairments that have been considered in the establishment of TLVs include those adverse responses that shorten life expectancy, compromise physiological function, impair a worker's ability to resist other toxic substances or disease processes, or adversely affect reproductive function or developmental processes. These and other criteria (e.g., prevention of infection) may need to be considered in the eventual establishment of exposure limits for biologically derived airborne contaminants.

In defining exposure limits for biological agents, a problem encountered with greater frequency than with many chemical substances is that biological exposures are often to complex mixtures of variable composition. Consequently, qualitative and quantitative information about exposure to biologically derived materials is often imprecise because agents other than those identified and measured may also be present and responsible for some of the health responses experienced by exposed persons. Given the constraints outlined above, at present, the usual approach of sampling workplace atmospheres and comparing measurements with TLVs cannot be applied to bioaerosols. In place of compliance monitoring, investigators can use the approaches outlined in this book and other references to assess and control exposures to biologically derived airborne contaminants.

1.3 Overview

This book is the fourth publication of the ACGIH Bioaerosols Committee to provide guidance on assessing and controlling bioaerosols in indoor environments (ACGIH, 1986, 1987, 1989). The book is divided into three parts: The Basics, Background Information, and Specific Agents. Following this introduction, Part I begins with a chapter on how to develop an investigation strategy. The subsequent chapters describe the primary health effects and symptoms that have been associated with bioaerosol exposure and outline a building walk-through inspection, the development of a sampling plan, sample analysis, and interpretation of environmental sampling data. Part II presents background information on the roles of medical professionals in investigations of bioaerosol-related illnesses, the transmission and control of respiratory infections, prevention and control of microbial contamination, collection of air and source samples for biological agents, data analysis, data evaluation, remediation of microbial contamination, and biocides and antimicrobial agents. Part III, on specific agents, begins with a chapter on the biology of the source organisms that may become airborne as bioaerosols or that may produce biologically derived airborne contaminants. The remaining chapters cover specific biological agents [i.e., bacteria, fungi, amebae, viruses, house dust mites, endotoxin and other bacterial cell-wall components, fungal toxins and β-(1→3)-*D*-glucans, antigens and allergens, and microbial volatile organic compounds]. Authors are not identified for the chapters in Part I because many committee

members and others contributed to these chapters. Primary and secondary authorship is recognized for other chapters.

Within chapters, individual sections are titled and numbered, the section numbers beginning with the chapter number. Section numbers are used throughout the book to direct readers to related discussions and explanations that appear elsewhere. These cross-references are set off in brackets. For example, the notation "[see 1.2]" directs readers to Section 2 in Chapter 1.

Many terms commonly shortened to acronyms and abbreviations are introduced once in the text, with later chapters using only the shortened form. Readers can find the full terms in the listing of acronyms and abbreviations beginning on page xi. The full terms for units of measurement that are abbreviated in the text are also listed for readers who may be unfamiliar with this notation. The Latin genus and species names of organisms are given on first use. Later references within a chapter adopt the standard convention of abbreviating the genus name to a first initial. A listing of genus and species names is provided on pages xi-xii for reference.

Occasionally specific products or tradenames are used in this book. Such mention does not imply endorsement on the part of an author or the publisher.

1.4 Approaches

We encourage users of this book to read Part I before beginning an investigation. We suggest that readers start with the chapters on investigation strategies and health effects. In addition to the information investigators can obtain through building inspections and environmental sampling for biological agents, the committee continues to feel that a health assessment is important for the successful handling of building-related illnesses that are due to bioaerosol exposures. A health assessment may be formal or informal, depending on the situation and circumstances, and does not always require the direct participation of medical professionals as members of an investigation team. Readers should note that the discussion of sample analysis precedes the discussion of sample collection. By presenting the information in this order, the importance is emphasized of considering what biological agents are sought, choosing an appropriate analytical method to identify and perhaps quantify them, and then identifying suitable collection methods.

The chapters in Parts II and III provide technical information that will aid industrial hygienists (IHs), environmental health professionals (EHPs), and indoor environmental quality (IEQ) consultants who conduct building evaluations. These chapters summarize the information needed to (a) understand when and why bioaerosols are important, (b) sample the environment to qualitatively or quantitatively determine if particular biological agents are present, and (c) prevent or mitigate problems related to bioaerosol exposure. ACGIH strives to provide accurate, complete, and useful information and the authors of this book have attempted to provide advice consistent with current knowledge on bioaerosol assessment and control. However, this is an area of active research and investigation and recommendations may change as newer information becomes available. Neither ACGIH nor any persons contributing to or assisting in the preparation of this information—nor any persons acting on behalf of these parties—makes any warranty, guarantee, or representation (express or implied) with respect to the usefulness or effectiveness of any information, method, or process disclosed in this material, nor do these parties assume any liability for the use of, or for damages arising from the use of, any information, method, or process disclosed herein.

The Bioaerosols Committee welcomes comments and suggestions on this book. Please communicate with the ACGIH Bioaerosols Committee at: ACGIH; Attn. Communications Dept.; Bioaerosols Committee; 1330 Kemper Meadow Drive, Suite 600; Cincinnati, OH 45240-1634; phone: 513-742-2020; fax: 513-742-3355; e-mail: comm@acgih.org.

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Chapter 2

Developing An Investigation Strategy

2.1 Introduction

2.1.1 Focus of This Book

The primary focus of this book is the identification and control of bioaerosol exposures in non-manufacturing workplaces, with an emphasis on the evaluation of actual or potential bioaerosol exposures in office environments. The approaches discussed here may also apply to institutional, commercial, and residential settings as well as some manufacturing and recreational environments. Of secondary concern in this book is the evaluation of biological contamination in the absence of human exposure, in which case investigations are conducted to assess and prevent material or structural damage due to biological contamination rather than to protect human health. The following chapters discuss the interpretation of air and source samples for various biological agents and the identification of bioaerosol exposures that may be associated with health complaints or disease. As discussed in Section 1.2, TLVs exist for certain substances of biological origin, however, ACGIH currently does not support any specific numerical guidelines for interpreting environmental measurements of (a) total culturable or countable bioaerosols (e.g., total bacteria or fungi), (b) specific culturable or countable bioaerosols (e.g., individual genera or species of microorganisms or classes of biological agents), (c) infectious agents, or (d) assayable biological contaminants (e.g., endotoxin (see 1.2.1 and 1.2.5), mycotoxins, allergens, or microbial volatile compounds).

The lack of exposure criteria for many bioaerosols precludes identifying excessive exposures solely by measuring air concentrations of biological agents. Information has been accumulated about the adverse health effects of some bioaerosol exposures from case studies of affected workers, epidemiological studies of groups of workers, and laboratory and clinical evaluations of humans as well as model *in-vivo* and *in-vitro* systems. However, the institution of workplace or ambient exposure limits for bioaerosols has been hampered by (a) the diversity of biological agents and their effects on individuals, (b) the often multiple agents to which workers are exposed, and (c) the lack of epidemiological or toxico-

logical data that establish dose–response relationships for carefully measured exposures to biological agents. In some cases, the lack of exposure data is due to the inadequacies of available sampling or analytical methods for the relevant biological agents. As a result, investigators identify associations between health effects and bioaerosol exposures on a case-by-case basis by combining health assessments with environmental observations and measurements.

2.1.2 Components Contributing to Bioaerosol-Related Illnesses

The strategies investigators use to study bioaerosol-related problems can play a role in how completely they understand what is happening in a work environment. Investigators strive to understand the interactions between a *host* (a worker) and an *agent* (a particular biological agent) that lead to contact and possibly an effect (Figure 2.1). In addition, investigators seek to explain how both of these factors influence and are influenced by the *environment* in which they meet (the workplace or building). Epidemiological studies often focus on identifying the time, place, and persons involved when assessing the effects of exposures to biological agents.

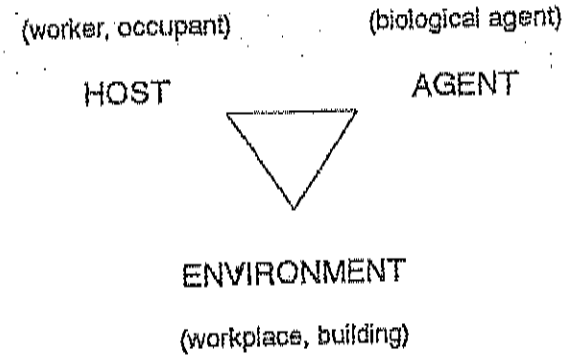

FIGURE 2.1. Fundamental components of bioaerosol-related illnesses.

For the discussion here, a *source* of a biological agent is the object, substance, animal, or person from which the agent was passed to a host. Typical indoor sources of biological agents are (a) people, who shed bacteria and viruses, (b) building materials, furnishings, and ventilation system components that provide a suitable environment for organism survival and growth, (c) accumulations of materials of biological origin on indoor surfaces, and (d) animals that shed allergens. Some texts use the term reservoir to describe indoor sites of microbial multiplication or accumulation. However, in this book, the term *reservoir* is used to designate the site in which an organism normally lives and multiplies and on which it primarily depends for survival. In some cases, the source and the reservoir of a biological agent are the same.

Typical reservoirs for biological agents are people, animals, plants, soil, water, and combinations of these. For example, a cooling tower may be the source of *Legionella pneumophila* that causes an outbreak of Legionnaires' disease in a building, but lakes, rivers, and streams are the natural reservoirs for this bacterium. Similarly, *Cladosporium herbarum* growing on damp walls in a building may be the source for worker exposure to fungal allergens, but the natural reservoir for this fungus is dead plant materials found outdoors. Distinguishing sources and reservoirs may be necessary when interpreting environmental sampling data and deciding how to control microbial growth. For example, *Legionella* spp. are frequently found in municipal water supplies because the bacteria may survive water treatment. Therefore, a one-time cleaning and disinfection of a warm-water storage tank will only temporarily rid it of the bacteria if they are likely to be reintroduced in the water supplied to a building. Conditions in the water storage tank (e.g., water temperature) must be maintained to minimize multiplication of these bacteria should they return.

Another familiar paradigm for understanding exposures to occupational hazards is to identify *sources*, *pathways*, and *receivers* (Figure 2.2). Separating the components of contaminant transmission in this way fits well with the scheme of identifying *hosts*, *agents*, and *environments* (Figure 2.1). Air is the common pathway through which the biological agents discussed in this book travel from sources to receivers. The respiratory tract is the primary target organ or site at which the effects of bioaerosol exposure are manifested, but systemic effects may also be seen as a result of the inhalation of some biological agents. Skin contact and ingestion are other potential routes of exposure to biological agents, but these exposure routes are addressed secondarily in this book.

2.1.3 Steps in an Investigation

Through training and experience, IHs, EHPs, and IEQ

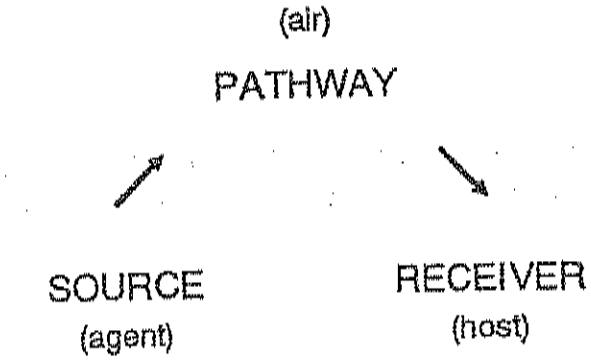

FIGURE 2.2. Paradigm for understanding and controlling occupational bioaerosol exposures.

consultants learn to gather and evaluate information about hosts, agents, and environments as well as sources and pathways. Experienced investigators use such information to reach supportable explanations for problems related to biological agents in indoor environments and to formulate reasonable recommendations to resolve problems. Figure 2.3 shows the main steps of this process, and Figure 2.4 explains in more detail how information about workers, biological agents, and buildings is combined in an investigation strategy. The following chapters of this book explain the implementation of these four steps (Table 2.1).

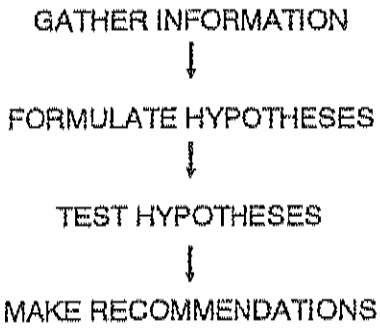

FIGURE 2.3. Fundamental steps in an investigation.

At each step in a workplace investigation, the participants should define their objectives and state their goals and expectations for the next activity. The overall objective of an investigation may change as further information becomes available, but the reasons for conducting an investigation should be defined as clearly and specifically as possible. The overall and specific purposes of a study should be agreed upon and communicated to the necessary parties as early as possible in the process.

Investigators can view these steps for assessing the presence and significance of biological and other agents in workplaces as similar to how physicians examine, diagnose, and treat patients. Some patients visit a doctor for a general checkup, others because they have particular concerns or complaints, and still others for serious problems that have developed. Likewise, workplaces may be evaluated on a routine basis, because certain conditions have arisen, or when clearly evident and potentially serious problems have become apparent. As for healthcare, measures to avoid bioaerosol-related problems may be categorized as primary, secondary, and tertiary prevention. First is prevention of worker exposure to potentially harmful biological agents. Good building design, operation, and maintenance could also be viewed as primary prevention in workplaces to avoid conditions

GATHER INFORMATION ↓	HEALTH ASSESSMENT. Determine if the symptoms occupants report appear to be building-related and if symptoms and building conditions could be associated with biological agents.
	BIOAEROSOL ASSESSMENT. Determine if the building's history suggests a potential for a bioaerosol problem. Identify biological agents that could be found in the workplace, as described, or that could cause reported symptoms.
	BUILDING ASSESSMENT. Inspect the worksite. Note damp areas, signs of water damage, potential sources of biological agents, and possible mechanisms for bioaerosol generation and transport.
FORMULATE HYPOTHESES ↓	THEORIES. Construct plausible hypotheses using available information about (a) occupant complaints and potential causes, (b) possible sources of biological agents, and (c) the building environment. Seek additional information from appropriate experts and published literature.
TEST HYPOTHESES COLLECT DATA ↓	ENVIRONMENTAL DATA. Collect and analyze relevant environmental information. Identify the fundamental factors responsible for the presence of biological agents.
	BIOAEROSOL DATA. As needed, consult bacteriologists, mycologists, entomologists, public health specialists, medical professionals, and toxicologists. Develop a sampling plan, and collect and analyze relevant air and source samples for specific biological agents.
	MEDICAL AND EPIDEMIOLOGICAL DATA. As needed, consult medical professionals, toxicologists, and epidemiologists. Collect and analyze relevant medical and epidemiological data.
INTERPRET DATA ↓	DATA ASSESSMENT. If evidence supports a hypothesis, proceed to identifying solutions. Otherwise, discard disproven hypotheses, formulate new ones, and collect data to test these.
MAKE RECOMMENDATIONS	REPAIR and PREVENTION. Repair causes of excess moisture and conditions contributing to the presence of biological agents. Clean contaminated materials and remove materials that cannot be reclaimed. Implement measures to prevent a recurrence of bioaerosol-related problems. Revisit site to confirm that control measures are effective.

FIGURE 2.4. Outline of an investigation strategy.

that allow bioaerosol entry or growth of biological agents in buildings. Second is intervention post-exposure, post-injury, or when workers have developed signs or symptoms of bioaerosol-related reactions. For building maintenance, prompt responses to signs of water intrusion or microbial contamination are common secondary prevention measures that limit adverse effects of such events. Last are measures designed to limit the consequences of evident problems (conditions either in workers or buildings) that have occurred through natural causes, accident, neglect, or unforeseen circumstances. Managers, supervisors, and health and safety professionals are responsible for early recognition of potential health problems in workers. Likewise, building operators and managers are responsible for primary and some secondary prevention in facilities under their supervision. Occasionally, these parties need outside advice to assess and determine how to address more serious problems related to microbial contamination or bioaerosol exposures or situations in which cause–effect relationships are difficult to establish.

2.1.4 Participants in Building Investigations

Investigators who study bioaerosol-related issues generally specialize in one of the three components outlined in Figure 2.1: (a) the health effects that the occupants (human hosts) experience, (b) the biological agents themselves and their detection, identification, and quantification, or (c) the environmental conditions that lead to the indoor presence of these agents and to workplace exposures. A doctor can better examine individual workers with building-related health problems if the treating physician also has information about workplace conditions.

TABLE 2.1. Outline of an Investigation Strategy and Related Chapters in this Book.

Investigation Step	Related Chapters	
Gather Information		
Health assessment	Ch. 3	*Health Effects Of Bioaerosols*
	Ch. 8	*Medical Roles And Recommendations*
	Ch. 9	*Respiratory Infections — Transmission And Environmental Control*
Bioaerosol assessment	Ch. 18	*Bacteria*
	Ch. 19	*Fungi*
	Ch. 20	*Amebae*
	Ch. 21	*Viruses*
	Ch. 22	*House Dust Mites*
	Ch. 23	*Endotoxin And Other Bacterial Cell-Wall Components*
	Ch. 24	*Fungal Toxins And β-(1→3)-D-Glucans*
	Ch. 25	*Antigens*
	Ch. 26	*Microbial Volatile Organic Compounds*
Building assessment	Ch. 4	*The Building Walkthrough*
	Ch. 10	*Prevention And Control Of Microbial Contamination*
Formulate and Test Hypotheses		
Collection of environmental data	Ch. 4	*The Building Walkthrough*
	Ch. 10	*Prevention And Control Of Microbial Contamination*
Collection of bioaerosol data	Ch. 5	*Developing A Sampling Plan*
	Ch. 6	*Sample Analysis* (also Ch. 17 to 26)
	Ch. 11	*Air Sampling*
	Ch. 12	*Source Sampling*
Collection of medical and epidemiological data	Ch. 3	*Health Effects Of Bioaerosols*
	Ch. 8	*Medical Roles and Recommendations*
	Ch. 9	*Respiratory Infections — Transmission And Environmental Control*
Data assessment	Ch. 7	*Data Interpretation*
	Ch. 13	*Data Analysis*
	Ch. 14	*Data Evaluation*
Make Recommendations		
Repair and prevention	Ch. 10	*Prevention And Control Of Microbial Contamination*
	Ch. 15	*Remediation Of Microbial Contamination*
	Ch. 16	*Biocides And Antimicrobial Agents*

On their part, microbiologists can better advise investigators on what environmental samples to collect and can interpret results more correctly if they know something about the environment under study and what, if any, health problems building occupants have experienced. Similarly, engineers and field investigators inspecting buildings should know what clinical diagnoses have been made for individual workers and what environmental samples have revealed.

Ideally, investigators studying different aspects of a building-related problem would consult one another and share information. Therefore, practitioners in different fields should be familiar with and appreciate the information and qualifications that other disciplines can contribute. Occasionally, investigators from the same or different disciplines may be asked to give their independent assessments of a situation. Rather than conduct an entire new investigation, consultants may also be asked to review a report prepared by others. An employer or building manager may give more weight to findings if multiple investigators reach the same conclusions and give similar advice on what needs to be done.

Investigators can be expected to assess buildings and building-associated complaints in accordance with the standard of care that applies to persons who are experts in conducting such evaluations. Investigators should act only within their areas of competence, as qualified by their education, training, experience, or demonstration of competency. Investigators should follow the standards of practice and comply with any restrictions pertaining to the practice of their profession, occupation, trade, or business. The lack of a title or degree does not mean a person is not qualified to conduct an investigation or render an opinion. However, possession of a title or degree does not automatically qualify a person as an expert. Only if they possess necessary permits, licenses, certifications, or registrations should investigators engage in activities that require such credentials.

Investigations of possible bioaerosol exposures often fall to IHs and EHP's because they are trained to identify work-related exposures and assess environmental conditions. This is logical because bioaerosol dissemination and the environmental conditions that contribute to the presence of biological agents are often the controlling factors in the host–agent–environment triangle (Figure 2.1). Membership in a professional organization indicates active participation in a field, but not all IHs or EHPs are trained or have experience in the investigation of bioaerosol-related hazards. IHs and EHPs can assist in such investigations by conducting site inspections, assessing exposures, and evaluating ventilation systems as well as by advising on the need for and proper selection and use of personal protective equipment.

Finally, investigators should undertake only those inspections, measurements, or tests for which they have or can obtain any necessary inspection, testing, or measurement equipment. Investigators should follow correct scientific procedures when conducting tests or making measurements and should only use methods that have been shown to be reliable. Investigators should avoid situations where they may encounter a conflict of interest that could impair or appear to impair their ability to provide accurate and unbiased assessments of health and safety conditions.

2.2 Gathering Preliminary Information

2.2.1 Health Assessments — the Host

The biological agents found in buildings can be diverse arising from plants, animals, or microorganisms. However, different biological agents may produce similar health effects. For example, some fungi, bacteria, and amebae cause infections often manifested by common initial symptoms of fever, chills, and malaise. Likewise the symptoms of hypersensitivity diseases are not specific to single causative agents even though the responsible antigen–antibody interactions are very specific. Inhalation fevers also have similar manifestations but many causes. Some reactions (e.g., asthma and rhinitis) may be caused by non-biological as well as biological agents. Therefore, although symptoms seldom identify the bioaerosols to which building occupants are exposed, the types of health effects observed may identify what category of agents investigators should seek (e.g., infectious agents, allergens, or causes of inhalation fevers).

Health assessments focus on potential hosts. However, it is not always possible to interview building occupants; indeed, some investigations are conducted to identify potential problems rather than to study building occupants who already have health or comfort complaints. Therefore, investigators may begin a study with a walkthrough site inspection and collection of data other than health data. Even in the latter case, a health assessment is not entirely missing from the evaluation process. The investigations discussed in this book are those that address the health and safety of current or future building occupants and how exposures to biological agents could affect these workers. Therefore, a starting point for any investigation is identification of the populations that might occupy a building or be exposed to potential bioaerosols, especially persons who may be highly susceptible to biological agents (e.g., very young or elderly persons, immunocompromised individuals, pregnant women, and persons with pre-existing medical conditions).

Health assessments determine whether conditions in a building could pose a hazard for occupants and if the symptoms that workers report are consistent with bioaerosol exposures. Chapter 3 describes the primary categories of reactions to bioaerosol exposures. Health assessments and occupant interviews need not be elaborate or time consuming activities but are often extremely

valuable for developing investigation strategies. Health assessments can help investigators narrow the focus of an investigation and identify the types of environmental information they need to collect. Investigators should consult a physician, other medical professional, or toxicologist—preferably one with experience in occupational and environmental medicine—to evaluate serious health complaints (e.g., asthma, possible hypersensitivity pneumonitis, and infectious diseases) [see 8.5.2].

Epidemiologists occasionally play important roles in investigations of bioaerosol-related health effects. Epidemiological studies establish case definitions for specific health complaints to identify affected and unaffected workers and compare their exposures [see 3.7 and 8.6]. For example, a study may find that previously well persons who recently began experiencing building-related allergy symptoms share a ventilation system and that complaints began after a humidifier was installed in the system. Epidemiological studies may also compare rates of particular symptoms among workers known to be exposed to a biological agent and matched control workers known not to be exposed. A conclusion that workers' symptoms are consistent with bioaerosol exposure warrants follow up with an on-site inspection to identify potential sources of biological agents and possible dissemination routes.

2.2.2 Bioaerosol Assessments — the Agent

Readers are encouraged to study the chapters in Part III, which provide information on the characteristics, ecology, reservoirs, sources, and health effects of the biological agents most commonly encountered in indoor environments. These include (a) living infectious microorganisms (e.g., bacteria, fungi, amebae, and viruses), (b) allergens from plants, microorganisms, arthropods, birds, and mammals, (c) endotoxin and other bacterial cell-wall components, (d) mycotoxins and β-(1→3)-*D*-glucans from fungi, and (e) microbial volatile organic compounds. Familiarity with the information in these chapters can help narrow the focus of a study, save valuable time and resources, and increase the likelihood that an investigation will accurately determine if biological agents are a potential source for concern. The fundamental questions regarding biological agents in indoor environments generally are not "Are biological agents present"—they almost certainly are, but "Is any biological agent present in an excessive amount," "Can building occupants be exposed to this material," "Would exposure to this agent cause adverse health effects," and "Could exposure to this agent account for building-associated health effects that building occupants have reported." Each chapter in Part III includes information on sample collection and analysis as well as data interpretation. Some of the chapters address remediation and control issues not covered in Chapter 10 or Chapter 15.

2.2.3 Building Assessments — the Environment

Chapter 4 describes how investigators use a site visit to survey building design and operation, look for potential sources of biological agents, and observe occupant activities. Inspectors should be accompanied by the building manager, maintenance personnel, and an employee representative, if available. Investigators may find potential sources of biological agents outdoors, in ventilation systems, or within the occupied spaces of buildings. Conclusions from an initial walkthrough inspection of a building generally fall into one of three categories. Investigators may (a) find no conditions that appear to contribute to the presence of unusual types or proportions of biological agents and conclude that excessive bioaerosol exposures are unlikely, (b) identify obvious problems and recommend solutions, or (c) note suggestive situations that warrant further evaluation.

2.3 Hypothesis Development

Investigators combine available environmental, epidemiological, medical, and toxicological evidence to develop hypotheses (i.e., carefully formulated logical answers or explanations). Many investigators make assumptions or guesses about problem situations without formally identifying these as hypotheses. Nevertheless, it is the pursuit of such theories that drives the investigative process. New evidence may fit neatly into a proposed scenario and strengthen a premise, or information may refute an explanation and convince an investigator to modify or discard a theory. Besides gathering information and formulating their own explanations, investigators should also listen to building managers and occupants. Ventilation system operators, maintenance personnel, workers, and supervisors often have theories about why their facility does or does not have problems. An investigator may not agree with the explanations these individuals offer, but often must address their concerns or include their theories with the hypotheses the investigator develops independently.

Following is an example of the type of hypothesis an investigator might use to guide a study. After conducting a preliminary site inspection and interviewing various parties, an investigator concludes that there appears to be fungal growth on ceiling panels in an office occupied by workers suffering from apparently building-related rhinitis. Specific subhypotheses in this theory are that (a) the discoloration and surface material on the panels is fungal growth, (b) sufficient moisture has reached this area of the ceiling to support microbial growth, (c) fungal spores from this growth are entering the breathing zone of the rooms' occupants, and (d) the symptomatic workers have developed sensitivity to allergens carried by spores of this specific fungus. Consideration of this example will be continued in Sec-

tion 2.4.4 to illustrate how investigators could proceed to test the validity of these assumptions as explained in the next section.

2.4 Hypothesis Testing

Reasons for the presence of biological agents and worker exposure thereto may be obvious in some situations, and investigators may proceed to formulate recommendations without confirming their hypotheses. Other situations may be less clear, more than one explanation may be plausible (requiring different resolutions), or substantiating evidence may be needed to warrant repairs or remediation. For these and other reasons, investigators often must test their hypotheses. Hypotheses are generally based on a series of assumptions that can be challenged. Investigators check their assumptions by devising tests that could disprove their theories. A hypothesis that survives challenge may be true [see 14.1.1].

2.4.1 Environmental Data

The proliferation of bacteria, fungi, protozoa, and arthropods in buildings depends on a number of interrelated variables. Moisture is recognized as the primary factor that limits biological growth. Chapters 4 and 10 describe what investigators look for when examining buildings for sources of biological agents. In particular, Section 10.3 describes different measurements of water in air and materials. Section 10.4 describes places investigators should look for indoor and outdoor moisture sources and potential sites of biological growth. Visual inspections and environmental assessments should be conducted by experienced persons. Examples of the tests they may perform are measurement of the relative humidity of indoor air; determination of mechanisms for moisture entry and vapor migration; measurement of pressure differences between indoor and outdoor zones or above-ground and subsurface spaces; conduction of tracer experiments to illustrate patterns of air movement and measure ventilation rates; examination of wall construction (e.g., the types of interior and exterior materials and the location of air and vapor retarders); and tests for rain entry, water drainage, and water wicking.

2.4.2 Bioaerosol Data

The lack of health-based exposure criteria for most types of biological agents precludes environmental sampling and simple comparison of measurements with established air concentrations and dose–response relationships [see 7.3.2 and 7.3.3]. Therefore, environmental samples are primarily collected to test suspected sources of biological agents, identify and quantify the agents present, and demonstrate bioaerosol release from environmental sources. A sampling plan includes specification of (a) the biological agents that are sought, (b) typical sources of the suspected agents, (c) the analytical methods that will be used to detect, identify, and quantify the agents along with constraints the methods may place on sample collection, (d) the sampling methods to be used, (e) the operating parameters of the sampling instruments, and (f) the locations and times at which samples will be collected [see 5.2].

Used properly, environmental sampling for biological agents can be a valuable investigative and research tool. However, inexperienced investigators are cautioned that such samples are unlikely to provide useful answers unless collected using a carefully considered sampling plan designed to address clearly stated, testable hypotheses. Scientifically sound quantitative sampling may be time consuming and costly. Therefore, investigators should carefully consider whether they need to sample for biological agents to understand the host–agent–environment and source–pathway–receiver interactions that are occurring in a particular setting in order to interrupt one or more of the links. Investigators on limited budgets can begin by designing an optimal sampling plan [see Chapter 5] and can then determine how much testing must be eliminated to stay within budget. At that point, the investigators must decide if the sampling they can afford to perform will be useful and defensible. If not, the investigators may need to adopt another approach to evaluate the situation or seek sufficient support to conduct a reasonable investigation.

2.4.2.1 Sample Analysis Although sample analysis follows sample collection, investigators should consider the kinds of analyses that will be performed before selecting a sampling method and collecting environmental samples. IHs, EHPs, and IEQ consultants who have little background in biological analyses are frequently unclear about what they can realistically learn from such testing. Participation of the analytical personnel in the hypothesis-generation and sample-planning phases of an investigation is strongly encouraged until investigators gain experience in conducting environmental assessments for biological agents.

Data interpretation problems (i.e., being unable to draw clear conclusions from environmental data) are more often the result of unclear objectives or poor planning in the study-design and sample-collection phases of an investigation than in the sample analysis portion. Analyses may be sophisticated, precise, and accurate without being useful if the reason for collecting samples was unclear, the sampling locations or times were poorly chosen, too few samples were collected to be representative, or the samples were collected improperly. For example, a laboratory may spend several weeks to carefully and correctly identify the many fungal and bacterial isolates that may be cultured from air samples. However, culture-based analyses identify only a small fraction of all microorganisms in environmental samples and critical bioaerosol exposures may go undetected if investigators rely solely on culture-based analytical methods. Chapter 6 provides an overview of what investigators can

learn from standard methods for analyzing various samples for biological agents. The sample analysis sections of the individual chapters in Part III can also guide investigators.

2.4.2.2 Sample Collection An investigator selects a sampling method by considering (a) the biological agents (or indicators thereof) of interest, (b) the analytical methods by which the laboratory will identify and perhaps quantify the material, and (c) the sites and times at which samples will be collected. Respectively, Chapters 11 and 12 describe air and source sampling. The chapters in Part III describe sampling for particular biological agents and their indicators or carrier particles.

The locations and times at which samples are collected depend on the hypotheses an investigation is pursuing. Chapter 5 discusses collection of representative samples. To identify building-related bioaerosol exposures, many investigators collect air samples using both spore traps and agar impactors, sampling outdoors and indoors at suspected problem and control locations. Settle plates do not collect airborne particles in a representative manner and do not reliably measure bioaerosol concentrations (see 11.3.1). Investigators should bear in mind that samples provide information about a site as it existed at the time tested. However, the findings may not represent conditions at a time in the past or future, even the relatively recent past or near future. Changes in the kinds, concentrations, and proportions of biological agents in the air can be rapid and substantial. Such fluctuations in undisturbed bulk and surface materials likely occur more slowly.

2.4.3 Medical and Epidemiological Data

The initial health-assessment phase of an investigation may identify a need for physical examinations or clinical testing of workers to diagnose their conditions, evaluate the severity of any illnesses, determine the work-relatedness of their problems, and recommend treatment. Preliminary interviews with workers may also indicate the value of a systematic and thorough collection of epidemiological data. Chapters 3, 8, and 9 describe these activities. Medical and epidemiological data are evaluated to identify supportable associations between bioaerosol exposures and health outcomes.

2.4.4 Example of Hypothesis Checking

This section returns to the example hypothesis presented in Section 2.3 to illustrate how to apply the concepts briefly outlined above. The investigators test the validity of their overall explanation by challenging each of their subhypotheses.

First, the investigators proposed that dark material on ceiling panels was fungal growth. They could check this assumption by inspecting the material more closely. Collection of adhesive tape samples could confirm that the discoloration on the ceiling was fungal growth and might even allow a presumptive identification of the fungus.

The second subhypothesis was that the ceiling was damp in the area of presumed fungal growth. Learning that the building's roof leaks during heavy rains would support this assumption. The observation of condensation on cold-water pipes above the affected ceiling panels would also explain how moisture could reach the ceiling. Use of a moisture meter or other device may provide an objective indication of the amount of moisture in materials. Measurement of dewpoint and surface temperature may shed light on the moisture dynamics of the space. Ultimately, the presence of microbial growth, which is only possible if water is available, provides evidence that sufficient moisture was present.

The third subhypothesis was that spores from the fungal growth became airborne for the office occupants to inhale. The investigators could collect air samples to see if spores of the fungus identified in surface samples were present at greater concentrations or as a greater fraction of the total air concentration in the room with visible growth than in other locations. The biological agents of interest are fungal allergens, but detection of fungal spores is often used to indicate allergen presence.

The final subhypothesis was that symptomatic occupants were immunologically sensitive to the fungus on the ceiling panels in their work area. Investigators could ask an allergist for advice on how to check this assumption and how helpful it would be to test building occupants for evidence of sensitivity or exposure to allergens from the fungus identified in surface and air samples. The evidence outlined in support of the first three subhypotheses should be sufficient to conclude that the source of excessive moisture in the ceiling should be stopped and the water-damaged ceiling panels should be replaced. However, for completeness, a medical evaluation of affected workers was also considered. In this example, a preliminary walkthrough inspection that included consideration of occupant symptoms led to (a) collection of environmental data and information about the building's history, (b) development of a surface and air sampling plan, followed by collection and analysis of environmental samples, and (c) possible medical and epidemiological evaluations of workers.

2.5 Data Assessments

Data assessment is the step where investigators make decisions on the relevance to human exposure of environmental observations and measurements, the strength of associations between exposures and existing or eventual disease, and the probability of current or future risks. Investigators frequently collect important information simply by observing physical evidence. An obvious interpretation of visible fungal growth in an area of chronic plumbing leaks is that undesirable conditions exist. Even

if exposure to airborne fungal spores or other materials has not yet occurred, it may in the future. In addition to observational data, investigators may also need to interpret data from environmental samples and epidemiological surveys.

Chapter 14 explains how investigators weigh evidence to reach decisions. Only investigators confident in their knowledge of biological agents, the methods used to collect and analyze samples for them, and the epidemiology of bioaerosol-related diseases should undertake interpretation of environmental sampling data. Occasionally, investigators need the support of medical professionals or toxicologists to conclude that measured exposures could produce the health effects observed or could pose a hazard for building occupants. Given the state of knowledge on some biological agents, such determinations may require a considerable research effort and be beyond the scope of an investigation. Such uncertainties may be especially problematic in legal deliberations [see 14.2.7]. A goal of data interpretation is to explain clearly how a host and an agent could come into sufficient contact in an environment to produce a particular effect. Other goals are explaining how biological agents enter buildings, identifying what environmental conditions have contributed to organism survival and growth indoors, understanding the pathways by which biological agents reach hosts, estimating the magnitude of exposures to biological agents, and justifying recommendations for correcting potentially or clearly harmful conditions.

2.6 Recommendations and Remediation

Investigators combine the information they have gathered from visual examinations, interviews, and testing to identify the hosts, agents, agent sources, and exposure pathways involved in current or potential problems (Figures 2.1 and 2.2). An understanding of these key features and their interactions can help investigators assess potential health hazards and identify actions that will minimize or prevent bioaerosol exposures that may lead to adverse health effects. Investigators base their conclusions and recommendations on their experience and professional judgement and should clearly communicate their findings, interpretations, and recommendations to the appropriate parties.

2.6.1 Sources

Not surprisingly, control of bioaerosol exposures focuses primarily on identifying environmental sources and interrupting biological processes. A concurrent focus is prevention of particle release from sources of biological agents. Section 10.2 discusses the factors that lead to biological contamination in buildings and how to control such problems. Chapter 15 outlines procedures to remove biologically contaminated building materials. Chapter 16 discusses disinfectants used to treat microbial growth and prevent its recurrence and cautions investigators against inappropriate use of biocides to treat microbial growth in building environments.

Other familiar forms of source control are water treatment to minimize microbial multiplication (e.g., amebae and *Legionella* spp.) as well as measures to minimize generation of water droplets that may carry infectious or allergenic agents [see 10.5 and 10.6]. Humidity control and good housekeeping—as measures to control mite and cockroach populations—are described in Sections 22.6 and 25.6. For infections spread from person to person (e.g., tuberculosis, colds, and influenza), early identification of affected workers and their removal from the workplace until appropriately treated or recovered are routine control measures [see 9.8.1].

2.6.2 Pathways

The exposure route from an identified source of a biological agent to a building occupant is often readily apparent. Still, investigators may be requested to demonstrate that a proposed exposure pathway exists by showing that the biological agent can be detected in indoor air. Occasionally, a means for worker exposure is unclear. For example, a building owner reluctant to undertake expensive repairs may argue that the hazard of microbial growth within a wall space is small if there is no obvious means for bioaerosols to penetrate the interior wall and reach building occupants. Likewise, for the earlier example of fungal contamination on ceiling panels, a building owner could argue that the contamination does not pose a hazard if there is no evidence that the fungal growth releases particles or volatile compounds. In such situations, an investigator's function may be to establish that a plausible or demonstrable exposure pathway exists. Investigators may be able to demonstrate that a proposed exposure pathway exists by showing that the biological agent can be detected in indoor air or by following particle transport or air movement using smoketubes, pressure differentials, or other means.

In some situations, biological agents may be known to be airborne, but their sources may not have been identified. Similarly, a known source may be in the process of remediation, and the investigators' responsibility is to minimize bioaerosol dissemination during the repair process. For such cases, investigators may limit exposures for building occupants and remediation workers by intercepting biological particles on their way from sources to receivers. Examples of means to interrupt agent transmission are the air filters used in large buildings to remove particulate matter from outdoor and return air. Section 15.2 describes the use of negative pressure and air filtration to contain bioaerosols during remediation operations. Section 9.8.6.4 mentions the use of filters to capture airborne pathogens and the use of ultraviolet germicidal irradiation to inactivate airborne infectious

agents. Drift eliminators on cooling towers are also designed to trap aerosols that might allow droplet-borne bacteria to escape in exhaust air [see 10.6].

2.6.3 Receivers

Controls that focus on the worker (i.e., the host or receiver) are a last resort in preventing bioaerosol exposures. Therefore, the use of personal protection (e.g., eye, skin, and respiratory protection) is only discussed as a precaution against bioaerosol exposure to be used during site inspections and clean up operations [see 4.6.2 and 15.2]. Some of these precautions are similar to control measures routinely used in microbiology laboratories as well as clinical and veterinary-care settings. For infectious agents, immunization of workers is an established means of protecting them from some airborne infectious diseases [see 8.2.3.4]. For hypersensitivity diseases, prevention of allergen exposure is preferred, but drug treatment may allow workers to tolerate some degree of workplace exposure, at least on a temporary basis [see 8.2.1.4]. Relocation or reassignment or workers may be necessary when exposure is unavoidable, drug treatment is unavailable or unacceptable, and use of personal protective equipment is impractical or unacceptable.

2.7 Risk Communication

Risk communication is too large a topic to cover adequately in this book, other than to emphasize its importance. Risk communication can be viewed as the formulation by experts of accurate, clear messages about the nature, magnitude, significance, and control of risk and the dissemination of this information to nonexperts. It might better be viewed as an interactive process through which individuals and groups exchange information and opinions. The primary factors that have been found to determine people's perception of risks are the fairness, familiarity, and voluntariness of their exposure. Deciding what level of risk is acceptable is not a technical question but a social, political, economic, and value-based one [see also 14.1.4.5].

Participants in building investigations asked to communicate with workers or building occupants can prepare by anticipating common questions and finding answers (or explanations for why the information is not available). Typical questions and concerns are (a) what agencies are involved in the investigation and which individual or group is in charge, (b) what hazardous agents are present and what are the potential consequences of exposure to these agents, (c) what kinds of samples have been collected and what did they show, (d) when will the investigation be completed and when will the audience learn the findings, and (e) in the interim, what is being done to ensure the workers' health and safety. Good communication can improve understanding, allay fears, lead to acceptance of information offered, and influence personal and organizational decisions, but poor communication can lead to confusion and distrust. Key to good communication of workplace risks are the credibility of the source of the information and the trust the audience has in this person or group.

2.8 Summary

This chapter provides an overview of the four steps involved in an investigation of actual or potential bioaerosol exposure (Figure 2.3). These steps represent an idealized approach and actual investigations may not proceed as systematically or be as complete. Therefore, investigators should be flexible and modify these steps on a case-by-case or as-needed basis. The time required for the various steps can vary greatly, depending on the conditions at the study site and the objectives of an investigation. However, an investigation should not be more time consuming or costly than necessary. When allocating resources, a balance should be sought between supporting a thorough investigation and reserving time and funds for remediation and prevention efforts that may be needed.

Investigations of bioaerosol-related hazards should aim to describe (a) what biological or other agents may be present, (b) the environmental conditions that may contribute to their presence, (c) how the agents may affect building occupants, (d) the means by which bioaerosols may reach humans, and (e) what can be done to prevent exposure. Unwary investigators should recognize that even experienced medical professionals, toxicologists, microbiologists, engineers, IHs, EHPs, and IEQ consultants often find it difficult to answer apparently straight-forward questions about biological agents, their effects on people, and the environments in which biological agents may be found. Therefore, investigators should draw on their own knowledge, experience, professional judgement and, above all, common sense during bioaerosol investigations and should recognize when they need assistance from other specialists. In addition, investigators should recognize the importance of effective communication with building designers, owners, operators, and occupants and the necessity and value of educating these parties about biological agents as well as their sources, potential health effects, and control.

Chapter 3

Health Effects Of Bioaerosols

3.1 Introduction

Building-associated complaints arise from diverse symptoms that workers experience as a result of exposures to various physical, chemical, and biological agents in buildings. The majority of health complaints in problem buildings are related to mucous membrane discomfort (i.e., eye, nose, and throat irritation), headache, and fatigue from unknown causes. The term sick building syndrome (SBS; or tight building syndrome) has been used to describe nonspecific building-related symptoms that cannot be associated with an identifiable cause. Although poorly defined, this term is still commonly used and widely understood as a distinction from specific building-related illnesses (BRIs), which are diagnosable diseases with known etiologies (Menzies et al., 1994; Hedge, 1995; Hodgson, 1995; Menzies and Bourbeau, 1997). To avoid possible misunderstandings associated with the term SBS, a distinction is made in this book between non-specific building-related symptoms (NSBRSs) and specific building-related illnesses (SBRIs). For simplicity, these terms have been shortened to BRSs and BRIs (Table 3.1). BRSs can be uncomfortable, even disabling, but permanent sequelae are rare (Redlich et al., 1997). Objective physiological abnormalities generally are not found, although there are several physiological markers for eye and mucosal effects (see 6.2). Specific BRIs occur less often than BRSs but some are more serious and all are accompanied by physical signs and laboratory findings.

Investigators who are familiar with the range of medical conditions associated with exposures to chemical, biological, and physical agents in indoor environments can design more efficient studies to identify the causes of workers' complaints. Investigators can also use this knowledge to predict problems that may arise as a result of exposure to indoor contaminants for which potential sources were observed during building inspections conducted in the absence of complaints. When appropriate, investigators not trained in medicine, epidemiology, or toxicology involve specialists in their studies. Thus, a health assessment, by a building investigator of any discipline, is part of the information gathering phase of the investigation process described in Chapter 2. As discussed in Section 8.1, medical expertise should be sought for (a) diagnosis and management of an individual worker with a possible BRI, typically building-related hypersensitivity disease, inhalation fever, or infection, and (b) investigation of populations of workers with BRSs. Table 3.2 is an alphabetical listing of the potentially bioaerosol-related health effects discussed in this book. This list covers those bioaerosol-related conditions seen in office, commercial, or recreational environments. Other health effects may be seen as a result of exposures to biological agents in health-care or laboratory settings, agricultural or animal-handling facilities, or manufacturing environments. This table lists known

causative agents and the respective chapters that discuss the conditions and the biological agents. Some of these conditions may also be seen as a consequence of exposure to chemical or physical agents, but Table 3.2 identifies only the primary biological agents that may be responsible for the listed conditions.

3.2 Building-Related Symptoms

3.2.1 Epidemiological Studies of Building-Related Symptoms

Work-related irritation of the mucous membranes of the eyes, nose, and throat are common in office workers, even in buildings not identified as having IEQ complaints (Nelson et al., 1995). Eye symptoms may include itching, mild redness, and irritation, and some building occupants may be unable to wear contact lenses in implicated buildings. Nasal symptoms include dryness, stuffiness, congestion, itching, and runny nose. Throat symptoms include feelings of dryness and irritation. Skin symptoms are less common, but workers may report dryness or skin irritation.

Specific causes of BRSs remain unclear, but exposure to biological and chemical agents have been implicated (see 3.2.2 and 26.3). It is often assumed that BRSs result from an insufficient supply of fresh (outdoor) air to an enclosed space. However, BRS complaints have usually not been found to correlate with ventilation rates (Robertson et al., 1985; Harrison et al., 1987; Skov and Valbjørn, 1987; Fanger et al., 1988; Menzies et al., 1993). An association has been noted for ventilation rates below 10 L/s (approx. 20 ft³/min) of outdoor air per person (Mendell 1993), but this is a fairly generous outdoor air supply rate and may not be achieved at all times in buildings operated by energy-conscious managers. European studies have found BRS symptoms to be associated with air-conditioned buildings with and without humidification (Finnegan et al., 1984; Burge et al., 1987; Skov and Valbjørn, 1987; Mendell and Smith, 1990; Mendell, 1993; Jaakkola and Miettinen, 1995).

Job category, job satisfaction, and gender also influence workers' perceptions of job-related symptoms. Further, occupant activities and building furnishings can affect IEQ and complaint rates. Specifically, the surface area of "fleecy" materials, exposed paper materials, and the amount and allergenicity of floor dust have been related to complaint rates (Gravesen et al., 1986; Skov and Valbjørn, 1987; Gyntelberg, et al., 1994). Buildings may have different reasons for poor IEQ, and a contaminated ventilation sys-

TABLE 3.1 Terms Used to Describe Building-Associated Medical Conditions

Sick building syndrome (SBS) (see Building-related symptoms)	
Building-related illness (BRI) or Specific building-related illness (SBRI)	Diagnosable illness whose cause can be directly attributed to exposure to an indoor chemical, biological, or physical agent. Medical condition of known etiology frequently accompanied by documentable physical signs and laboratory findings: • Infection (e.g., acute viral infection, legionellosis, or tuberculosis), • Syndrome associated with exposure to a chemical or physical agent (e.g., carbon monoxide poisoning, dermatitis from glass fibers, or irritant-induced or exacerbated asthma or rhinitis), • Immunologically mediated disease (e.g., allergic rhinitis, sinusitis, asthma, or hypersensitivity pneumonitis), • Inhalation fever associated with exposure to a biological agent (e.g., humidifier fever, Pontiac fever, or other febrile, flu-like illness).
Building-related symptom (BRS) or Nonspecific building-related symptom (NSBRS)	Nonspecific symptom (e.g., eye, nose, or throat irritation, headache, fatigue, or other discomfort) that usually cannot be associated with a well-defined cause but that appears to be linked with time spent in a building.

TABLE 3.2. Alphabetical Listing of Conditions Related to Exposure to Biological Agents and Chapters in which the Conditions and Agents are Discussed

Condition	Description	Ch.	Known or Suspected Causative Agents	Ch.
Allergic bronchopulmonary mycosis (ABPM)	A condition seen in asthmatics resulting from immunological reactivity to colonization of the airways with a fungus (see Allergic broncho-pulmonary aspergillosis).	25	Fungi (e.g., *Penicillium* spp.)	19
Allergic bronchopulmonary aspergillosis (ABPA)	The most common form of allergic broncho-pulmonary mycosis in which the colonizing fungus is an *Aspergillus* sp.	25	*Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus terreus*	19
Allergy	An immune-mediated hypersensitivity to a foreign material (see Hypersensitivity diseases).	3, 8, 25	–	–
Asthma	A chronic inflammatory condition of the airways; usually characterized by intermittent episodes of wheezing, coughing, and difficulty breathing.	3, 8, 25	Bacterial antigens Fungal allergens Amebic allergens Arthropodal allergens Bird, pollen, or mammalian allergens Endotoxin	18, 25 19, 25 20 22, 25 25 18, 23
Bronchitis	Inflammation of the mucous membranes of the large airways, characterized by excessive mucus production and cough.	8	–	–
Building-related illness (BRI)	Diagnosable illness accompanied by documentable physical signs and laboratory findings that is associated with indoor exposure (see also Infectious diseases, Hypersensitivity diseases, and Inhalation fevers).	3, 8, 9, 25	(see specific health effect)	–
Building-related symptom (BRS)	Nonspecific symptom (e.g., eye, nose, or throat irritation, headache, fatigue, or other discomfort) that cannot be associated with an identifiable cause but that appears to be linked to time spent in a building.	3, 8, 25	Endotoxin Mycotoxins β-(1→3)-*D*-glucans MVOCs	18, 23 19, 24 19, 24 26
Common cold	Head cold, acute viral rhinitis, acute coryza; characterized by rhinitis, sneezing, lacrimation (tearing), irritated nasopharynx, chills, and malaise (see also Influenza).	8, 9	Rhinoviruses or coronaviruses	21
Conjunctivitis	Inflammation of the mucosal surface of the eye surrounding the cornea; characterized by lacrimation (tearing), irritation, and redness; seen with some hypersensitivity and infectious diseases and in response to certain biological and chemical irritants.	25	–	–
Cryptococcosis	Systemic fungal infection usually presenting as a respiratory infection and occasionally as meningitis (e.g., in HIV-infected persons).	3, 9, 15	*Cryptococcus neoformans*	19
Dermatitis	Inflammatory skin condition, skin reaction, skin rash; seen with some hypersensitivity and infectious diseases and in response to contact with certain biological and chemical agents (see also Toxic effects).	25	–	–

TABLE 3.2. Alphabetical Listing of Conditions Related to Exposure to Biological Agents and Chapters in which the Conditions and Agents are Discussed (cont.)

Condition	Description	Ch.	Known or Suspected Causative Agents	Ch.
Granulomatous amebic encephalitis (GAE)	Rare inflammation of the brain due to infection with an ameba	20	*Acanthamoeba* or *Balamuthia* spp.	20
Hantavirus pulmonary syndrome (HPS)	Often fatal acute respiratory infection preceded by a flu-like illness.	3, 9	Sin Nombre virus (SNV) or Four Corners virus (FCV)	21
Histoplasmosis	Systemic fungal infection of varying severity, with the primary lesion usually in the lungs.	3, 9, 15	*Histoplasma capsulatum*	19
Humidifier fever	A form of inhalation fever; characterized by fever, chills, muscle aches, malaise, and chest symptoms.	3, 8	Bacteria (GNB) Amebae Endotoxin	18 20 18, 23
Hypersensitivity disease (allergy)	Disease for which a subsequent exposure to an antigen produces a greater effect than that produced on initial exposure (see Allergy, Allergic bronchopulmonary aspergillosis, Allergic bronchopulmonary mycosis, Asthma, Hypersensitivity pneumonitis, Rhinitis, and Sinusitis).	3, 8, 25	Bacterial antigens Fungal allergens Amebic allergens Arthropodal allergens Bird, pollen, or mammalian allergens	18, 25 19, 25 20, 25 22, 25 25
Hypersensitivity pneumonitis (HP) (extrinsic allergic alveolitis)	An inflammatory disease of the lung due to cell-mediated immunological reactions in pulmonary tissues.	3, 8, 25	Bacterial antigens Bacterial proteases Fungal allergens	18, 25 18 19, 25
Infection	Entry and multiplication of an infectious agent in the body, the result of which may be inapparent or result in an infectious disease (see Infectious disease).	—	—	—
Infectious disease	Clinically manifest disease resulting from an infection (see Common cold, contagious disease, Cryptococcosis, Granulomatous amebic encephalitis, Hantavirus pulmonary syndrome, Histoplasmosis, Influenza, Legionnaires' disease, Primary amebic meningoencephalitis, Pneumonia, and Tuberculosis).	3, 8, 9	(see specific infection)	—
Influenza	Acute infectious disease of the respiratory tract; characterized by fever, headache, myalgia, prostration, rhinitis, sore throat, and cough.	8, 9	Influenza virus	21
Inhalation fever	Febrile, flu-like illness following heavy exposure to certain biological or chemical agents from environmental sources (see Humidifier fever and Pontiac fever).	3, 8	Bacteria, legionellae Fungi Amebae Endotoxin	18 19 20 18, 23
Legionnaires' disease	Bacterial pneumonia; preceded by anorexia, malaise, myalgia, and headache; accompanied by fever, chills, and (occasionally) cough.	3, 8, 9	*Legionella* spp., most often *Legionella pneumophila*	18, 15
Opportunistic fungal infection	Fungal respiratory infection primarily seen in immunocompromised persons.	3, 8, 9, 15	Fungi	19

TABLE 3.2. Alphabetical Listing of Conditions Related to Exposure to Biological Agents and Chapters in which the Conditions and Agents are Discussed (cont.)

Condition	Description	Ch.	Known or Suspected Causative Agents	Ch.
Organic dust toxic syndrome (ODTS)	Poorly characterized condition similar to humidifier fever; short-lived, febrile reaction following exposure to high organic dust levels (see Inhalation fever).	3, 8	–	–
Pneumonia	Lung infection; characterized by filling of the alveoli with secretions, cough, chest pain, shortness of breath, and fever.	3, 8	Bacteria Viruses	18 21
Pontiac fever	A form of inhalation fever; characterized by fever, chills, headache, and myalgia.	3, 8	*Legionella* spp.	18, 15
Primary amebic meningoencephalitis (PAM)	Rare inflammation of the brain and its lining due to infection with *Naegleria fowleri*.	20	*Naegleria fowleri*	20
Rhinitis (coryza)	Inflammation of the lining of the nose causing rhinorrhea (runny nose), congestion, itching, and sneezing due to either allergy or direct irritation.	3, 8, 25	(see specific health effect)	–
Sick building syndrome (see Building-related symptom)				
Sinusitis	Inflammation of the mucosal lining of the sinuses leading to purulent nasal and pharyngeal drainage and cough (see Rhinitis).	3, 8, 25	–	–
Sore throat	Inflammation of the mucous membranes and underlying parts of the nasopharynx, pharynx, and larynx; associated with pain or discomfort on swallowing (see Common cold, Influenza, and Building-Related Symptom).	3, 8	(see specific health effect)	–
Toxic effect	Reaction to a biological toxin; may involve various organ systems (e.g., inhalation fever, mucous membrane or skin irritation, dizziness or tremors, nausea, immune suppression.	3, 18, 19, 23, 24, 26	Bacterial toxins Mycotoxins MVOCs	18, 23 19, 24 26
Tuberculosis (TB)	Bacterial infection that may affect many organs but most commonly the lungs.	3, 8, 9	*Mycobacterium tuberculosis*	18

tem is sometimes a source for complaints (Fanger et al., 1988). Thermal discomfort and perception of humidity or dryness may also be associated with symptoms (Menzies et al., 1993; Sundell and Lindvall, 1993; Jaakkola and Miettinen, 1995; Nelson et al., 1995). Mendell (1993) summarized the epidemiological literature pertinent to the effects of building, workspace, job, and personal factors. These data showed consistent associations between increased symptoms and the following building characteristics: air conditioning, carpets, occupant density, and video display terminal use. The following personal characteristics have also consistently been associated with increased symptoms: female gender, job stress or dissatisfaction, and a history of allergies or asthma. NIOSH looked for associations between environmental factors and work-related health conditions in 80 complaint office buildings and found increased relative risks associated with presence of debris inside the air intake, poor or no drainage from drain pans, presence of suspended ceiling panels, and recent renovation with new gypsum board (Sieber et al., 1996). It is unclear from cross-sectional studies whether the association between BRSs and allergies/asthma represents an overlap of symptoms or whether either of these conditions predisposes persons to the other.

3.2.2 Bioaerosols and Building-Related Symptoms

Bioaerosols have not been conclusively associated with BRSs, although biological agents are strong contenders in etiologic hypotheses. Burge et al. (1987) and Finnegan et al. (1984) suggested that correlations of BRSs with humidifica-

tion and cooling were due to microbial contamination. BRSs have been associated with fungal multiplication and the presence of unusual types of fungi (Morey et al., 1984; Morey, 1988). The chief candidates for relationships between BRSs and bioaerosols are endotoxins (from Gram-negative bacterial cell walls), mycotoxins (from certain fungi), and other microbial components or products [e.g., β-(1→3)-*D*-glucans and microbial volatile organic compounds (MVOCs)], which later chapters discuss in detail. Investigators have targeted these bioaerosols because their effects, theoretically, would not depend on immunological sensitization and might be sufficiently brief to account for complaints that resolve when occupants leave a building. In this regard, reports associating BRSs with endotoxin and perhaps β-(1→3)-*D*-glucan exposures offer promising leads requiring confirmation and further study (Rylander et al., 1992; Gyntelberg et al., 1994; Teeuw et al., 1994).

Many hypotheses about hosts, agents, and their environments must be considered when investigating particular problem buildings. Potential contributors to BRSs include unsuitable ventilation system design, inadequate ventilation rates, improper ventilation system maintenance, and offgassing of volatile compounds from building materials and furnishings as well as emission of a multitude of airborne substances from occupant activities, microbial growth, and the accumulation of dust containing biological agents. Section 8.3 discusses physiological tests physicians use to assess building-related health effects and medical management of affected workers.

3.3 Hypersensitivity Diseases

Hypersensitivity diseases result from exposure to antigens, which stimulate a specific immunological response [see Chapter 25]. Hypersensitivity pneumonitis (HP) is associated with antigen-specific immunoglobulin G (IgG), elevated T lymphocytes in bronchoalveolar lavage fluid (Fink, 1983), and granuloma on lung biopsy. Other antigen-mediated diseases [e.g., allergic rhinitis (or "hay fever")] and most allergic asthma occur in persons with a genetic makeup that allows production of antigen-specific IgE.

Attack rates of hypersensitivity diseases among exposed building occupants are usually low, because an immunological reaction is required for these diseases to occur. However, even one clearly building-related HP case constitutes a significant public health event because the disease can be so devastating. Such a diagnosis should trigger a search for additional cases and initiation of corrective measures to reduce or eliminate bioaerosol exposures. HP and elevated asthma risk can co-exist in problem buildings (Hoffman et al., 1993). Most building-related antigens are assumed to be of fungal or bacterial origin, but protozoa have also been implicated. Dust-mite and animal allergens are common causes of allergic asthma in the residential environment and, to some degree, in workplaces (Lundblad, 1991; Janko et al., 1995).

3.3.1 Rhinitis and Sinusitis

Allergic rhinitis and sinusitis are diagnosed by patient history, physical examination, eosinophils in nasal smears, immediate skin-prick tests to aeroallergens, elevated total or specific IgE antibody levels, and occasionally by specific nasal challenge [see 8.2.1.1 and 25.2.2.1]. Building-related allergic rhinitis may be common and may overlap with BRS complaints but has been poorly documented (Robertson and Burge, 1985). Building-related allergic rhinitis is often accompanied by conjunctivitis. Future investigation of building-related allergic rhinitis or sinusitis would likely be most successful among office workers at risk for HP or asthma because antigens that cause these diseases might also elicit allergic nasal and sinus responses.

3.3.2 Asthma

Building-related asthma is characterized by complaints of chest tightness, wheezing, cough, and shortness of breath that are worse on work days and improve on weekends or vacations. Symptoms may occur within an hour of exposure, onset may be delayed for 4 to 12 hours, or both. To diagnose asthma, physicians rely on patient history, wheezing, and either reversible air flow limitation or elicitation of air flow limitation on challenge with low doses of pharmacological agents (methacholine or histamine) [see 8.2.1.2 and 25.2.2.1]. A work-related pattern of reduced peak air flows often indicates that asthma is due to a building-related exposure. Patients diagnosed with building-related asthma should be restricted from problem environments to prevent worsening of the condition, although remission may not occur in cases of long-standing exposure. In addition to exposure cessation, patients can be treated with asthma medications such as bronchodilators, corticosteroids, cromolyn, or theophylline.

Building-related asthma in office workers is not commonly recognized. Some case reports have shown asthma to be associated with humidifier use. Biocides used in humidification systems have been suspected as a cause of office-associated asthma (Finnegan and Pickering, 1986). Exacerbation of asthma from bioaerosols generated from a home cool-mist humidifier has been established using quantitative sampling and bronchial provocation testing (Solomon, 1974). Epidemic asthma has also occurred in a printing plant in association with a contaminated humidifier (Burge et al., 1985). A five-fold increased risk of endemic asthma was observed for occupants of an office building with moisture incursion from a below-grade wall (Hoffman et al., 1993). Asthma has also been seen in the industrial setting in high prevalence among workers with elevated daily endotoxin exposures from contaminated water sprays (Milton et al., 1995). In this industrial setting, it is likely that much of the asthma was due to irritant rath than allergic mechanisms.

3.3.3 Hypersensitivity Pneumonitis

HP is characterized by (a) acute, recurrent pneumonia with fever, cough, chest tightness, and lung infiltrates, (b) a progression of cough, shortness of breath, fatigue, and chronic lung fibrosis, or (c) an intermediate pattern of acute and chronic lung disease. Industrial hygienists are familiar with many types of HP in workers exposed to organic dusts. For example, farmers, pigeon breeders, cheese makers, wood processors, and mushroom growers are all at risk for HP (Rose, 1994; Rylander and Jacobs, 1994; Yang and Johanning, 1997). However, HP may also occur in various office, residential, and recreational environments.

Physicians diagnose HP on the basis of patient history and the following clinical signs or symptoms (a) abnormal chest radiograph or lung computerized tomography (CT) scan, (b) abnormal respiratory sound (rales) heard through a stethoscope, (c) reduced pulmonary function including reduced diffusing capacity, (d) pattern of exercise-tolerance-test findings compatible with interstitial lung disease, (e) lymphocytic alveolitis on bronchoalveolar lavage, (f) granulomas and interstitial fibrosis on lung biopsy, (g) reproduction of symptoms and signs with bronchial challenge with sterile extracts of implicated environmental organisms, or (h) reduction of symptoms and signs with corticosteroid medications or removal from bioaerosol exposure [see also 8.2.1.3 and 25.2.2.3]. A clinician should consider the public health implications of building-related HP and the possibility that a person is an index case when deciding whether to proceed to bronchoscopy to make a definitive diagnosis for patients with building-related chest symptoms (in whom asthma has been excluded) even in the absence of radiographic disease. Persons with building-related HP may require permanent restriction from entering implicated environments because, once sensitized, individuals react to extremely low, often unmeasurable, concentrations of antigenic material.

Precipitins (i.e., precipitating antibodies, usually IgG) to common saprophytic organisms or to an extract of material collected from an implicated environment may be present in the blood of exposed building occupants, even if they are not symptomatic [see 25.2.3.1]. Presence of these precipitins can provide a justification for collecting environmental samples to measure antigen exposures (Reed et al., 1983; Reed and Swanson, 1986). Often the specific biological antigen (e.g., the specific microorganism involved) in an HP outbreak or its precise source [Reed et al., 1983; Kreiss and Hodgson, 1984; Hodgson et al., 1985, 1987] not known, although there are exceptions (Woodard et [1988]). In the latter case, only one fungus was isolated from environmental samples and corresponded with specific precipitins in symptomatic individuals.

Investigation of building-related HP outbreaks has been reviewed elsewhere (Kreiss and Hodgson, 1984; Kreiss, [1989]). Other publications have attributed HP to bioaerosols from water incursion and water-damaged furnishings (Hodgson et al., 1985; Hoffman et al., 1993), contaminated air-handling units (Bernstein et al., 1983; Hodgson et al., 1987; Woodard et al., 1988), and water sprays in indoor pools (Rose et al., 1998).

3.4 Inhalation Fevers

3.4.1 Humidifier Fever

Humidifier fever is a flu-like illness, characterized by fever, chills, muscle aches, malaise, and chest symptoms [see also 8.2.2.1]. These symptoms usually arise within 4 to 8 hours of exposure and subside within 24 hours, usually without long-term effects. People with humidifier fever rarely consult physicians for this short-lived, flu-like illness and, if they do, seldom undergo extensive diagnostic testing. Consequently, the pathophysiology of this condition is unclear, as it may overlap with radiologically normal HP. Recent work suggests that intermittent, high exposure to endotoxin may cause humidifier fever, whereas daily endotoxin exposure may result in asthma (Milton et al., 1995). In addition, repeated, severe episodes of inhalation fever due to endotoxin exposure may lead to emphysema. Thus, some cases of humidifier fever may be toxic diseases rather than diseases resulting from immunological responses. Organic dust toxic syndrome (ODTS) is a poorly characterized condition similar to humidifier fever. ODTS is diagnosed when short-lived, febrile reactions follow exposures to high dust levels in composting and various agricultural operations.

3.4.2 Pontiac Fever

Legionella spp. are associated with two BRIs: Legionnaires' disease and Pontiac fever. The former is a bacterial pneumonia [see 3.5.1]. The latter is a self-limited, flu-like illness characterized by fever, chills, headache, and myalgia. Pontiac fever was first described in 1968 in a 144-case Michigan epidemic in a county health department building where the attack rate was nearly 100% and the average incubation period was 36 hours (Kaufmann et al., 1981). Contaminated air-conditioning systems, whirlpool spas, steam turbine condensers, and industrial coolants have been associated with outbreaks of Pontiac fever (Friedman et al., 1987). Pontiac fever is diagnosed by an elevated serum antibody titer but is not considered to be an infection because live bacteria have not been recovered from clinical cases and the disease has apparently been caused by non-culturable bacteria (APHA, 1995) [see also 8.2.2.2]. Why these bacteria cause two distinct clinical syndromes has been attributed to (a) the inability of some legionellae to multiply in human tissue (for a variety of reasons, including virulence, host range, and viability of the bacteria), (b) unusual characteristics of their lipopolysaccharides and possible toxic effects of dead bacteria (Kaufmann et al., 1981; Horwitz, 1993), and (c) differences in host susceptibility (Fields, 1997).

3.4.3 Other Diseases from Irritant or Toxic Exposures

Conjunctivitis, rhinitis, and asthma may occur by irritant as well as immunological hypersensitivity mechanisms (Kipen, et al., 1994). For example, asthma has been observed in the industrial setting in high prevalence among workers with elevated daily endotoxin exposures from contaminated water sprays (Milton et al., 1995). In this industrial setting, it is likely that much of the asthma was due to irritant rather than allergic mechanisms. Building-related exacerbations of pre-existing allergic rhinitis or asthma may be triggered by exposure to irritant or toxic compounds, and these exposures may have a significant impact on disease severity (Michel et al., 1996).

Cancer occurs in clusters among office workers as it does in other occupations and among residential neighbors. Environmental cancer generally has a long latency period (i.e., the disease does not appear until years after the responsible exposure has occurred). Clusters of cancers of the same cell and organ type are rare and have not been clearly linked with indoor air pollution. Although it does not present in work-related clusters, radon-associated lung cancer is also an indoor air risk (Pershagen et al., 1994). Other carcinogens in indoor air to which workers may be exposed include asbestos, certain mycotoxins (e.g., aflatoxin, fumonisin, ochratoxin, patulin, and sterigmatocystin) (see 24.2), and environmental tobacco smoke.

The potential for toxigenic fungi to cause pulmonary hemorrhage and death in susceptible individuals (infants) has received much recent attention (CDC, 1995a; Jarvis et al., 1996; Sorenson et al., 1996; Montaña et al., 1997). However, the evidence to support a plausible exposure route and inhalation of a significant dose of mycotoxin remains weak. Exposures to agents other than mycotoxins were also significantly associated with the single reported outbreak (CDC, 1995a; Burge, 1996; Montaña et al., 1997). Cancer has been associated with ingestion of mycotoxins, but the only association of cancer with inhalation exposure to mycotoxins has been in heavily contaminated agricultural environments (see 24.2.4.1).

To date, no studies have been conducted to investigate the role of mycotoxins in the development of cancer for persons exposed in non-agricultural indoor environments. The toxic endpoints (i.e., various types of cancer) and the potency of carcinogenic mycotoxins have been characterized in animal and a few human studies. Currently available methods for measuring indoor exposures to mycotoxins do not yet allow a conclusion as to whether sufficient exposure occurs in non-agricultural settings to initiate or promote cancer (see 24.2). On the other hand, some bioaerosols are known to reduce the risk of cancer. Epidemiologic and experimental studies have demonstrated that high levels of endotoxin exposure may be protective against lung cancer. However, no studies have been conducted of cancer prevention and endotoxin exposure in residential or office environments (see 23.2).

3.5 Infectious Diseases

The term *infection* is used to describe the entry and multiplication of a biological agent in a host's body. An *infectious disease* is a clinically manifest reaction to an infection (see 9.3.2). The term *infectious* may also mean able to transmit an infectious agent, for example, an infectious person is one who not only is infected but is also able to transmit the responsible agent to others. Infectious diseases resulting from agents transmitted primarily from person-to-person are called contagious diseases.

3.5.1 Legionnaires' disease

Legionnaires' disease is a bacterial pneumonia most often caused by *Legionella pneumophila* and first recognized in a 1976 outbreak that resulted in 182 cases (29 fatal) among Legionnaires attending a convention at the Bellevue Stratford Hotel in Philadelphia. Since then, epidemic and sporadic cases have been associated with buildings and traced to aerosols from cooling towers and evaporative condensers as well as sprays from whirlpools, shower heads, supermarket vegetable misters, and potable water supplies. There is evidence that not all cases are due to airborne transmission of the bacteria and that drinking contaminated water followed by aspiration is the route of exposure in certain cases (see 9.4.2). *Legionella* spp. are ubiquitous in nature. The incubation period for this pneumonia is 2 to 10 days, and only a small percentage (~5%) of exposed persons contract the disease. In addition to pneumonia, the infection can produce gastrointestinal tract, kidney, and central nervous system symptoms. Legionnaires' disease is diagnosed as outlined in Section 8.2.3.1.

3.5.2 Other Infectious Diseases

The risk of contagious disease transmission in buildings with low outdoor air ventilation rates or high rates of air recirculation has attracted much public interest. Outbreaks of tuberculosis, varicella (chicken pox), measles, and smallpox have confirmed the importance of airborne disease transmission (LaForce, 1986). Measles epidemics have spread by aerosol transmission through ventilation systems (Riley, 1980), and epidemic pneumococcal disease has been documented in an inadequately ventilated jail (Hoge et al., 1994). Swiss investigators found that absenteeism due to respiratory illness was greater in a fully air-conditioned building than in a naturally ventilated one housing a similar population (Guberan, 1985). Brundage et al. (1988) reported increased rates of febrile respiratory infection in army trainees housed in modern, tightly sealed barracks compared with trainees in older, draftier barracks. Military personnel in Operation Desert Shield

who slept in tight, air-conditioned buildings (which were preferred and therefore crowded) complained more often of sore throat and cough (Richards et al., 1993). Troops who slept in naturally ventilated tents complained more often of rhinorrhea, possibly due to higher exposures to outdoor air pollutants and allergens.

Unusual infections sometimes appear as building-associated epidemics in environments such as hospitals and research laboratories. For example, the Q-fever agent (*Coxiella burnetii*—a rickettsia) has been disseminated through ventilation systems in buildings housing infected sheep, goats, or cattle and in buildings in which the organism was being grown in a laboratory. Q-fever symptoms include high fever, chills, headache, and myalgia and sometimes pneumonia, hepatitis, and endocarditis. Systemic fungal infections (e.g., histoplasmosis and cryptococcosis) have occurred when contaminated bird droppings or construction/demolition dusts were disseminated in indoor environments (Rose, 1994; CDC, 1995b). The viral agent of hantavirus pulmonary syndrome (the Sin Nombre or Four Corners virus) may be spread by inhalation from infected rodent droppings and urine (Jackson, 1994). Finally, many opportunistic bacterial and fungal pathogens can cause infectious disease in immunocompromised persons occupying buildings where these common organisms are present [see 8.4].

3.6 Other Building-Related Symptoms

Bioaerosols are often suspected as causative agents for BRSs when other causes are not readily identified even if, in fact, another cause is responsible. For example, detergent residues left in carpets after cleaning can cause cough and dry throat symptoms (Kreiss et al., 1982; CDC, 1989) and, though rare in office environments, carbon monoxide poisoning can cause headache, fatigue, and nausea.

Persons with BRSs whose complaints are not addressed and whose exposure persists sometimes develop a more general awareness of or sensitivity to other environments. These reactions may continue despite improvements in the initial environment (Rosenstock and Cullen, 1994). Such persons may also find that their symptoms become more severe with time and do not resolve after leaving the problem building or environment as they did initially. Over the last 20 years, an increasing number of such patients have been labelled as having environmental illness or multiple-chemical sensitivity (MCS), a syndrome with a variety of definitions (Fiedler and Kipen, 1997). Although several etiologic theories have been postulated for MCS, no agreement has been reached on diagnostic criteria for or causes of such reactions. In fact, some affected persons may have developed asthma or irritant or allergic rhinitis with chronic sinusitis that has gone unrecognized and untreated. Whatever the proximate cause, it seems clear from clinical experience that an important contributing factor in the development of these chronic conditions is a delayed response to complaints of BRSs and a sense on the part of the affected workers that management does not believe or value them.

Early investigations of BRS complaints occasionally resulted in a diagnosis of mass psychogenic illness (mass hysteria) when specific causes were not readily found to explain symptoms. Certainly, the occupants of buildings with high complaint levels are often angry and fearful after encountering managerial resistance to investigations, inconclusive results from studies, or ineffectual remedies. However, the diagnosis of psychogenic illness has specific criteria, as do most other medical diagnoses (Guidotti et al., 1987), and this diagnosis is not appropriate merely because other causes are not apparent. Mass psychogenic illness is characterized by subjective symptoms with features difficult to explain on an organic basis. Hyperventilation is common, and epidemics are transmitted from person to person via a visual or verbal chain (Baker, 1989; Boxer, 1990). Incidence rates of mass psychogenic illness may shift throughout an occupied space and rarely correlate with measurable air quality parameters. Chronic health complaints related to IEQ are unlikely to be due to mass psychogenic illness, even though a psychologic overlay is common. Poor management, boring work, incorrect lighting, temperature variations, uncomfortable work stations, and noise may lower peoples' complaint thresholds. However, air quality and thermal comfort complaints are likely to have a preventable basis, which can often be determined from epidemiological evidence and environmental inspection.

3.7 Epidemiological Investigations

Epidemiology is the study of the occurrence and distribution of diseases and other health-related conditions in populations (Kelsey et al., 1996). Many epidemiological studies of building-associated problems are *descriptive stud-*

TABLE 3.3. Components of an Epidemiological Study

- Define what constitutes a case of illness (i.e., establish a case definition).
- Consider possible confounding factors (i.e., variables that are related to both risk and outcome, for example, the presence of multiple antigens in a contaminated environment).
- Select appropriate control groups (i.e., unexposed or unaffected workers).
- Carefully design a questionnaire that can be administered with or without an interviewer.
- Select and administer appropriate diagnostic tests to screen for or confirm disease or condition.
- Determine how data will be analyzed before collecting data.

ies, intended to provide information on patterns of symptom occurrence in populations according to various characteristics such as work activities and location, worker age and gender, and onset and timing of symptom occurrence. *Analytical studies* test hypotheses and are carried out when leads about the causes of a condition are already available. Examples of analytical investigations are case–control studies and cohort studies, which may be prospective or retrospective. Of these study designs, common epidemiological approaches include investigations of the prevalence of health and environmental conditions in problem environments as well as comparisons of cases and controls within problem environments or in apparent problem and non-problem areas.

An epidemiological investigation can often clarify whether there is a building-related problem and, if so, its nature as well as possible means for resolution. Mapping the distribution of cases in time and space within a building may lead to hypotheses regarding probable sources and pathways and may suggest appropriate sampling protocols. Table 3.3 outlines the components of a well-designed epidemiological study.

Generic questionnaires are available for investigating IEQ complaints [see 8.5.2]. Using a standardized questionnaire facilitates comparison of findings with other studies. For example, the USEPA and NIOSH have an IEQ questionnaire and have compiled a database derived from investigations that used the questionnaire (http://www.cdc.gov/niosh/ieqwww.html). However, investigators may decide to tailor a questionnaire to gather information that will enable them to test a specific hypothesis not studied previously. A good questionnaire avoids leading questions and eliminates ambiguities. Information on risk factors (e.g., contact lens use, pre-existing medical conditions, and smoking habits) may be necessary, as well as demographic information, identification of health complaints (with their time of onset and occurrence), and location of respondents within a building. Information on possible exposures outside the work environment may be pertinent for some types of complaints. Precoded forms facilitate data analysis in investigations involving large numbers of people.

Medical epidemiologists, often in collaboration with other specialists, can arrange appropriate diagnostic testing to substantiate or further define medical problems suggested by questionnaires. However, objective tests are not available for BRS outbreaks, except in a research context. For hypersensitivity diseases, pre- and post-shift spirometry, peak flow measurements, diffusing capacity measurements, chest radiographs, and serum precipitin testing can be used, with clinical referral of suspected cases for more definitive diagnostic tests [see 8.6.1].

A major concern for epidemiologists is measurement, for example, measurement of exposures, diseases, confounding variables, and effect modifiers (Kelsey et al., 1996). Epidemiologists need to carefully choose the most reliable sources of data, the correct study design, representative study populations, and the appropriate methods of measuring the parameters of possible importance. Unfortunately, epidemiological investigations are difficult in many workplaces and other indoor settings because the numbers of cases and populations available for study are often small and the methods available to measure exposures to biological agents may be limited. Additionally, it may be difficult to categorize workers into exposure categories if their jobs require them to spend time in more than one part of a building or to engage in multiple activities. Comparisons between populations from separate work areas or buildings have been conducted, but this approach is not always possible and, when it is, investigators may encounter problems if they cannot eliminate possible confounders or measure exposures adequately.

3.8 Summary

Medical assessment of occupants with IEQ complaints is an ideal way to begin an investigation. A study to confirm that a biological agent is the cause of a BRI (e.g., a hypersensitivity disease, inhalation fever, or infection) may differ substantially from a search for the cause of BRS complaints. Admittedly, physicians, epidemiologists, and toxicologists are rarely involved in investigations from the beginning. However, other disciplines can approach building-related complaints using the conceptual framework outlined here. Once the causes of building-related complaints are understood and corrected, epidemiological surveillance tools can be used to demonstrate that the problem has been resolved or to document its reappearance. Even in the absence of complaints from building occupants, investigators can use information about known associations between biological agents and health outcomes to judge the safety of conditions and practices they observe in buildings.

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Chapter 4

The Building Walkthrough

4.1 General Considerations

4.1.1 What Investigators Can Learn from Walkthrough Inspections

On-site building inspections are conducted for many reasons, among them (a) to assess the suitability of a space for a particular group of occupants and type of activity, and (b) to attempt to identify possible causes of complaints from current building occupants. During preliminary building walkthroughs, investigators collect environmental information of a fairly general nature [see Figure 2.4]. A preliminary inspection may identify the need for an in-depth building evaluation, during which investigators focus on potential problem areas and gather data to address specific questions. Obtaining evidence of worker exposure to biological agents and proving causal associations between specific exposures and symptoms is often difficult. However, a thorough walkthrough inspection by a team of experienced investigators may yield valuable insight about the existence of sources of biological agents and possible pathways from sources to building occupants [see 2.1.2].

4.1.2 Conducting a Walkthrough Inspection

During initial walkthroughs, investigators collect primarily observational data (i.e., information obtained by visual inspection of a building and interviews with the building operator and occupants). Investigators (a) examine the physical structure, maintenance activities, and occupancy patterns of a building, (b) look for potential sources of biological agents, (c) look for evidence

of current or past water damage or excess moisture, (d) note sources of other indoor air contaminants, and (e) as needed, formulate plans for an in-depth investigation or for control and remediation of noted problems. Building management, heating, ventilating, and air-conditioning (HVAC) system operators, maintenance personnel, and employee representatives should accompany investigators on building walkthroughs.

Sources of biological agents may be found (a) outside buildings, (b) in attics and below-grade spaces, (c) within wall cavities, (d) in HVAC systems (Figure 4.1), and (e) in the occupied space. Investigators should obtain and study building blueprints, if available, including those for any retrofitting and remodeling that has been done. As-built plans, which incorporate changes made during construction, can be important to identify where deviations from the original building design occurred and where problems may arise. Although seldom available or complete, maintenance and reconstruction records can provide valuable information regarding the history and current status of HVAC system operation.

4.1.3 Areas Frequently Associated with Biological Contamination or Bioaerosol Entry

Table 4.1 summarizes specific sites and equipment that investigators may check and some of the problems they may find while conducting a building walkthrough. Appendix 4.A provides a sample checklist that emphasizes identifying sources of biological agents. Investigators should view the example checklist as a companion to Chapters 10 and 15, which identify areas where biological growth often occurs in buildings and what can be done to avoid and correct problems. The table and appendix are coded by letter to correspond with the HVAC system components shown in Figure 4.1.

4.1.4 Moisture and Other Factors

In addition to the features highlighted in Table 4.1 and Appendix 4.A, investigators should note moisture anywhere in a building that may have supported biological growth in the past or may do so in the future. Temperature must also be considered, because of its role in moisture transfer and condensation (see 10.2). The use of portable, hand-held moisture meters may enable investigators to pinpoint areas of potential biological growth during a walkthrough inspection. Many of these meters detect the presence of moisture by measuring the electrical conductance between two probes inserted into the medium to be tested. When set to the proper resistance for the material to be tested, a moisture meter can give a reliable indication of the amount of moisture present. Foarde et al. (1996) used a moisture meter in a number of field investigations and found that the meter detected significant moisture in building materials when moisture was not otherwise evident to the investigators. In several of these studies, the investigators found biological growth associated with these damp areas as well as reports of symptoms by the building occupants. Moisture meters can be used to survey the moisture associated with any non-conductive porous material to which the probes can be applied (e.g., ceiling tiles, gypsum board, and carpeting) (Foarde et al., 1996). These devices indicate internal moisture content, a difficult measurement to make without a meter. Moisture meters provide qualitative information but do not indicate the actual amount of water available to microorganisms for growth, as defined by

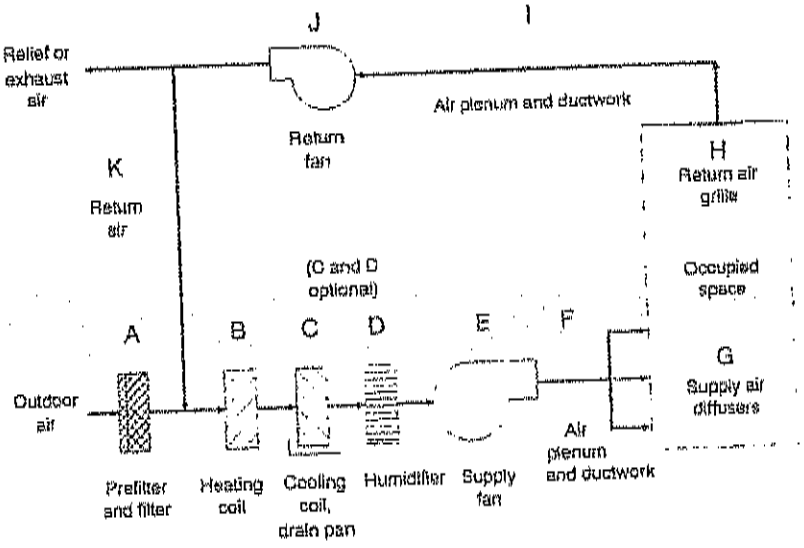

FIGURE 4.1. Typical HVAC system components for a large building.

water activity, a_w, [see 10.3]. Other instruments use radiowaves to detect temperature and moisture differences remotely (e.g., at wall surfaces).

Identification of a source of a biological agent does not necessarily implicate bioaerosols as a substantial factor contributing to occupants' illness or complaints. Even in assessments of suspected biological contamination, investigators should consider other reasons for poor air quality and occupant dissatisfaction with the indoor environment. Various documents can aid investigators in conducting systematic building walkthrough inspections (AIHA, 1990; USEPA/NIOSH, 1991; USEPA, 1994; Morey, 1995; ISIAQ, 1996). Godish (1995) describes several protocols for investigating problem buildings.

4.2 Outdoor Sources of Bioaerosols

Microorganisms are normal inhabitants of the outdoor environment. Outdoor air often contains fungal spores,

TABLE 4.1. Potential Sources of Biological Agents or Bioaerosol Entry Routes into Buildings and Factors Related to Microbial Growth or Bioaerosol Dissemination (letters in parentheses identify components illustrated in Figure 4.1)

Source/Route	Related Factors
Outdoors	
Outdoor air	Crop planting and harvesting—exposed soil, soil turning, or disturbance of plant materials; excavation or construction operations; wastewater treatment or irrigation; textile mills; slaughterhouses or rendering plants; composting operations
Building exterior	Poor grading or water drainage; evidence of water intrusion (discoloration); blocked rain gutters; penetrations in siding or veneer; damage to building envelope; wood rot in structural timbers; animal infestation near building or in crawl space beneath building; automatic lawn sprinklers that wet exterior walls
HVAC System	
Outdoor air intakes (OAIs)	Bioaerosol sources near OAIs (e.g., plant debris, feathers and bird droppings, insect or rodent infestations, sanitary air vents, cooling towers or evaporative condensers, standing water); below-grade OAIs
Filters (A)	Dampness; microbial growth on filters; gaps between filters and housings; low efficiency filters
Heat exchangers (B and C)	Dirty heating or cooling coils; excessive water in condensate pans—inadequate drainage from collection pans; blow-through of water droplets onto surfaces downstream of coils; dampness and microbial growth on acoustical lining; poorly maintained air washers or humidifiers; stagnant water in air washers or humidifiers
Supply air plenums and ductwork (F)	Excessive surface deposits; dampness and surface microbial growth; inaccessible humidifiers
Supply air diffusers (G)	Surface deposits, rust, or microbial growth on louvers; soiling of adjacent ceilings and walls; poor air mixing
Occupied Space	
Water-damage	Evidence or history of plumbing or roof leaks, water intrusion or spills, high indoor humidity (>70%), attempts to clean or disinfect carpets and other materials, musty or moldy odors
Chronic condensation	Inadequate insulation or intrusion of humid outdoor air that results in chronic condensation on windows, perimeter walls, or other cool surfaces
Window air conditioners and evaporative air coolers	Location inconvenient for maintenance; dirty grilles; standing water in condensate pans or sumps; dampness and surface microbial growth in or near units
Fancoil and induction units	Dirty heating or cooling coils or filters; excessive water in condensate pans—inadequate drainage from collection pans; dampness and surface microbial growth near units
Potted plants	Microbial growth on leaves, soil, plant containers, or surfaces in contact with containers; excess moisture from overwatering
Carpet	Poorly maintained or water-damaged carpet that serves as a source for dirt accumulation or microbial growth
Fabric office partitions, wall coverings, drapes; upholstered furniture	Poorly maintained or water-damaged fabric-covered and upholstered items that serve as sources for dirt accumulation or microbial growth
Portable (console) humidifiers	Poorly maintained units with microbial growth in the water reservoirs; spray or mist units
Return air plenums (I)	Excessive surface deposits; dampness and surface microbial growth

pollen, bacteria, and algae as well as fine plant and insect fragments (Muilenberg, 1995). Fungal spores may account for a few percent to approximately a quarter of particulate matter less than 10 μm (PM10). Pollen can also be a significant component of outdoor air on a mass basis. Except during periods of snow cover, fungal spores and bacteria are abundant in outdoor air, and levels may increase dramatically during and immediately following rain events. In addition to knowing about regional factors that contribute to the atmospheric bioaerosol load, it is essential that investigators evaluate the neighborhood immediately surrounding a building. Possible local sources of strong bioaerosol emissions include farming, animal handling, composting, and certain industrial operations. Examples of outdoor bioaerosol sources that may be immediately adjacent to a building are cooling towers, standing water on roof tops, and accumulated organic matter (e.g., leaves, grass clippings, feathers, bird or bat droppings, insect colonies, and rodent nests). Bioaerosols from such sources, once disseminated into the ambient air, may find their way into buildings through OAIs, windows, doors, vents, and inadvertent openings such as joints and cracks in the envelopes of negatively pressurized buildings.

4.3 The HVAC System

4.3.1 HVAC System Design

Ventilation systems in large buildings mix outdoor and recirculated air, heat or cool this mixture, and distribute it to the occupied space (Figure 4.1). Ventilation systems in residences and similar small buildings usually have simpler means to condition indoor air and typically do not provide for mechanical intake of outdoor air. Rather, these systems circulate indoor air through heating or cooling units and distribute the air through ductwork or directly into a space. In some cases, no mechanical circulation is provided and the indoors is heated radiantly. In such buildings, window evaporative coolers or air conditioners may be used for cooling. In some climatic regions, humidifiers of various types are used to add moisture to dry indoor air. Where indoor humidity conditions are too high, dehumidifiers may be used to remove excess water. The finding of a humidifier or dehumidifier in a building should prompt investigators to inquire why it is needed. To diagnose problems in HVAC system performance, building inspectors must be familiar with the climatic conditions of the geographic area in which a building is located as well as what building designs, construction materials, and HVAC equipment are appropriate for the region.

4.3.2 Outdoor Air

4.3.2.1 Outdoor Air Supply Rate An inadequate supply of outdoor air often leads to building-related complaints and symptoms, regardless of the presence or absence of inappropriate bioaerosols (Seltz, 1990). A properly maintained and operated mechanical ventilation system can reduce indoor bioaerosol concentrations by limiting infiltration of outdoor bioaerosols and by diluting those from indoor sources (Parat et al., 1994, 1996). The American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE, 1989) has recommended a minimum of 10 L/s (20 ft³/min) of outdoor air per person for office environments. Provided the outdoor air being introduced is of high quality and properly conditioned, the introduction of adequate volumes of outdoor air may improve indoor air quality by reducing the concentration of indoor contaminants through dilution [see 10.5.2]. Residences and similar small buildings rely on natural ventilation for outdoor air entry (i.e., open windows and doors and cracks in the building envelope). Closing and sealing these openings can make heating and cooling more efficient, but may reduce outdoor air infiltration to levels too low to adequately dilute indoor contaminants. Opening windows immediately increases the outdoor air supply, but allows penetration of outdoor aerosols and can waste energy and be uncomfortable.

4.3.2.2 Outdoor Air Quality The location of the OAI can significantly affect the nature of outdoor bioaerosols that enter a building (Bearg, 1993) [see 10.5.4]. A bioaerosol source with a clear pathway to an OAI may also result in contamination of the interior of an HVAC system. Rooftop OAIs are vulnerable to bioaerosol sources such as cooling towers, sanitary vents, building exhausts, and standing water. Streetlevel and below-grade OAIs are susceptible to entrainment of moisture, vehicle emissions, and other ground-level pollutants including odors and dust from decaying vegetation and accumulated animal matter.

Cooling towers and evaporative condensers located close to or directly upwind of OAIs should be evaluated as potential bioaerosol sources, especially in legionellosis investigations [see 10.6]. Films, foam, and algae on wet surfaces may indicate poor maintenance. Water samples and scrapings of biofilms can be collected from these water reservoirs for analysis. Usually such analyses are only necessary where specific BRIs (e.g., Legionnaires' disease, Pontiac fever, or HP) have been identified and specific biological agents are suspected.

Stagnant water, soil, and plant and animal matter near or in an OAI can support the growth of bacteria and fungi which can subsequently enter a building. In addition, birds and bats may use OAIs, window ledges, eaves, and attics as roosting and nesting sites. Accumulated bird or bat droppings may harbor the infectious fungi *Cryptococcus neoformans* and *Histoplasma capsulatum* as well as other fungi and bacteria. Similar problems can occur where large flocks of birds nest or roost in trees near occupied buildings. Rodent and other animal in-

festations near OAIs are also of concern [see 9.2.1]. Investigators may collect bulk samples of material near OAIs if necessary to confirm the biological nature or content of the material.

4.3.3 Filtration

A primary function of filters within HVAC systems (A in Figure 4.1) is to protect heating and cooling coils (B and C). Filters may also reduce maintenance requirements by limiting the accumulation of dirt throughout a building and its HVAC system and may contribute to worker health by removing potentially harmful particles, including ambient aerosols and those from local sources. However, such particles may enter an HVAC system and possibly penetrate to the occupied space if a filter has poor efficiency for fine particles or if there are gaps between the filter and the filter housing (Pasanen, 1995). HVAC system filters are not intended to protect equipment and occupants from heavily contaminated air such as may enter an OAI located near a strong outdoor bioaerosol source [see 10.4.4.1 and 10.5.3].

Filters may become damp during the air-conditioning season or when an OAI is not adequately protected from rain, snow, or fog. Microorganisms may grow on a damp filter itself or on collected dust (Martikainen et al., 1990; Kemp et al., 1995; Morey, 1996; Parat et al., 1996; Simmons et al., 1997). Microscopic analysis of material accumulated on filters may help distinguish between a normal accumulation of material of biological origin on a filter and actual microbial growth.

4.3.4 Heat Exchangers

In many large buildings, the mixture of outdoor and return air passes a heat exchanger, which generally consists of heating and cooling coils (B and C in Figure 4.1). Heat and moisture are either added to or removed from the supply air to maintain comfortable conditions. During the air-conditioning season, water condenses from warm air as it passes over cooling coils. This condensate collects in a drain pan beneath the coils and should promptly exit the air-handling unit through drain lines (Figure 10.7). If present, humidification devices are located downstream of the heat exchanger (D in Figure 4.1). In some large buildings, cooling may be accomplished in part by passing air through a water spray (air washer) (Morey, 1988). Section 10.4.4 describes problems investigators may find with heat-exchange equipment.

4.3.4.1 Surface Condensation within HVAC Systems An increase (up to 90%) in the relative humidity (RH) of air downstream of cooling coils is a natural result of the energy transfer between the air and the coils. Moisture may condense on cool surfaces in contact with this damp air or may "wick" off cooling coils. Particles (e.g., soil, organic matter, and microorganisms) not removed by the filtration system can collect on surfaces within HVAC systems. Such organic matter may support microbial growth under wet or damp conditions [see 10.4.4.2]. In these areas of HVAC systems, materials that retard water evaporation or provide increased surfaces on which dirt can accumulate may present particular problems. Investigators may sample surfaces in HVAC systems if microbial contamination is suspected and needs to be confirmed.

4.3.4.2 Drain Pans and Standing Water Microorganisms can grow in the stagnant water that results when drain pans are inadequately pitched toward an outlet or a drain is blocked (Morey et al., 1984) (C in Figure 4.1). The pressure differential between the inside and outside of a ventilation system may also affect water drainage [see 10.4.4.3 and Figure 10.7]. The presence of a film or foam in standing water may indicate microbial growth or other contamination. Investigators may collect water samples for testing if they suspect and need to confirm microbial growth. However, interpretation of test results requires experience because such waters will always yield some culturable bacteria and fungi. In addition, investigators must consider if there is a mechanism for dissemination of contaminants into the occupied space. Microorganisms may release odorous compounds, which can readily be distributed throughout a building, but the release of particles from contaminated water requires mechanical disturbance or other action sufficient to produce aerosols.

4.3.4.3 Air Washers and Humidifiers Sumps for air washers and humidification devices that use recirculated cold water are always microbially contaminated to some degree (D in Figure 4.1) [see also 10.4.4.4]. Because these devices generate aerosols, they should always be considered potential bioaerosol sources. Growth may also occur in a heat-exchange plenum if the surfaces are sufficiently cool to allow condensation. Steam humidification systems have been shown to emit fewer bacteria and fungi than water spray systems (Burkhart et al., 1993), but condensation and related microbial growth may still be a problem. Samples can be collected from air washers, humidifiers, and damp surfaces to confirm contamination and to identify contaminating organisms.

4.3.5 Supply Air

Conditioned air leaving a heat exchanger is transported to the occupied spaces of a building through a system of ducts (F in Figure 4.1). In large buildings, the main supply air plenum or duct is usually constructed of sheet metal, which may be internally or externally insulated for noise control and to minimize heat exchange. Air from the main supply air plenum travels until it enters a branch duct or a terminal (Morey and Shattuck, 1989). Air eventually

enters the occupied space through diffusers which, if properly designed, promote mixing of the conditioned supply air with room air [see 4.4.1].

4.3.5.1 Supply Air Plenums and Ductwork Supply air ducts are often internally lined with glass fiber to reduce noise and/or to insulate so as to minimize heat exchange with surrounding materials. Any surfaces within supply air plenums and ductwork can accumulate dirt that, in the presence of adequate moisture, can serve as a substrate for microbial growth. Although ductwork is never sterile or entirely free of debris, it should not be coated with a thick layer of deposited material. For example, glass-fiber ductwork should be undamaged and should show its original color and metal ductwork should still look metallic.

If dirt and debris have accumulated on ductwork, microbial growth can occur when sufficient moisture becomes available [see also 10.4.4.5]. Humidification devices may be located in supply air ductwork (rather than near a heat exchanger) where they cannot easily be reached for cleaning or examination. The reservoirs of these humidifiers are often contaminated, and microorganisms may grow on downstream ductwork if water condenses there [see 4.3.4.3].

Investigators can collect surface samples of ductwork deposits suspected of being microbial growth. Collection of samples from apparently clean ductwork is less important, but may provide laboratory personnel with a reference against which to judge other samples. Identification of the same biological agent in an HVAC system and in air in the occupied zone may implicate the ventilation system as the source or disseminator of the contaminant. However, many of the fungi and bacteria that grow as contaminants within ventilation systems are also common in outdoor air. Therefore, investigators should compare the types and relative concentrations of microorganisms in indoor and outdoor air to identify whether the biological agents arise from outside the building (in which case, better filtration may be needed) or if the source for the air contaminants is within the HVAC system.

4.3.5.2 Supply Air Distribution The distribution of conditioned air to occupied spaces may also disseminate airborne particles. Particles may originate from inadequately filtered outdoor air, contamination within an HVAC system, or contaminants from the occupied space that enter the return air [see 4.5]. Thus, an HVAC system that moves air and particles throughout a building may become a major distribution pathway for both chemical and biological agents. In a "healthy" indoor environment (i.e., one with no unusual sources of biological agents), bioaerosol concentrations vary as a function of outdoor bioaerosol concentration, HVAC system characteristics, the number of building occupants present, and the activities conducted in the building.

4.4 The Occupied Space

4.4.1 Supply Air Diffusers

Conditioned air enters the occupied space through supply air diffusers (G in Figure 4.1), which are either the terminations of a ductwork system or openings from a pressurized ceiling plenum. Contaminant concentrations and relative humidity rapidly come to equilibrium when conditioned air mixes with room air. For example, during the air-conditioning season, comparatively dry air from a diffuser reduces the overall RH in a room. Likewise, the addition of clean, conditioned air reduces the concentration of pollutants generated within an occupied space.

Particles entrained in the supply air stream can also deposit on a diffuser's louvers and on adjacent ceiling and wall surfaces. Particles from the occupied space may also be entrained in eddy currents induced by the supply air stream. These currents travel along the ceiling, walls, and floor and then back into the diffuser air stream from which the dirt may deposit on adjacent surfaces. Often, this soiling is only a cosmetic concern. However, in air-conditioned buildings, cold air leaving a diffuser may cool adjacent surfaces below the dew point, and the resulting condensation may support microbial growth. Investigators may collect samples of such deposits for microscopic examination to differentiate soil and soot deposits from microbial growth.

4.4.2 Fancoil and Induction Units

Fancoil and induction units may handle a significant portion of the heating and cooling load in a large building (Morey and Shattuck, 1989). These units are located in enclosures, usually beneath the windows in perimeter areas or offices or in columns in interior spaces. Fancoil units contain a fan, heating and cooling coils, and a filter with which they recondition and recirculate indoor air. Induction units mix a portion of room air with conditioned air provided from a central air-handling system. Like central ventilation system components, fancoil and induction units are susceptible to dust accumulation and may become sites for microbial contamination. If condensate collection pans in these units do not drain properly, humidity may become sufficiently high that dirt on filters and other surfaces can support microbial growth. In addition, the water may overflow onto floors, carpets, and wall systems.

4.4.3 Water Leaks, Spills, and Elevated RH

Important water sources in the occupied building zone are plumbing and roof leaks, water spills and overflows, and high RH [see Figure 10.2]. Water-damaged materials provide sites for microbial growth (e.g., ceiling tiles, wall coverings, carpet and other floor coverings, wickerware, upholstered furniture, books and paper, as well as leather and wood items) (ISIAQ, 1996). Section 10.3 describes different measures of moisture in air and in building mat

rials and how researchers use these measurements to identify when microbial growth can occur.

The microclimate at exterior wall surfaces is distinct from the more homogeneous climate of a building's interior walls. The transfer of heat between indoor air and exterior walls or windows will produce condensation if the dew point is reached [see 10.4.3]. This water then becomes available to microorganisms on these surfaces (Lstiburek, 1994; Morey, 1995; IEIAQ, 1996). Often, water damage and resultant microbial growth are obvious. If necessary, suspected growth can be confirmed by collecting surface or bulk samples for examination under a microscope.

4.4.4 People, Animals, and Plants as Sources of Biological Agents

People themselves are sources of viruses (e.g., the agents of influenza and the common cold) and of human-source bacteria. Therefore, the more densely occupied a building, the greater workers' exposure to such microorganisms. Some pathogenic viruses and bacteria are spread by coughing and sneezing [see 9.2.2], and many bacteria are shed with skin sloughed off by abrasion (e.g., by clothing rubbing against skin) [see 14.2.3.2 and 18.5.2].

Animal occupants of buildings (e.g., cats, dogs, rodents, cockroaches, and other arthropods) shed allergens. Indoor plants may also harbor pests, and moisture from overwatering may support microbial growth. Therefore, investigators should note the presence of animals and plants indoors. People may also carry particles into a workplace on their clothing (e.g., animal danders) [see 25.5.4]. In cases where allergies are suspected, but sources cannot be identified, workers should be questioned about contact with animals outside the workplace. Consistently elevated RH (especially within porous materials) can support dust-mite populations. Carpeting installed on uninsulated concrete floors at ground level is of particular concern because of the potential for dampness, condensation, and both microbial and dust mite growth. Dust samples collected with vacuum devices can be tested for dust-mite and other common allergens (e.g., cockroach, bird, cat, and dog antigens) as well as microorganisms [see 22.4 and 25.4].

4.5 Return Air

In most office and institutional buildings, air exits from the occupied space by passing through a common return plenum or an open space above a suspended ceiling (I in Figure 4.1). Air in the common return plenum enters the main return air duct and is transported back to an air-handling unit for reconditioning (K in Figure 4.1). In some buildings, air in the occupied space enters branch return ducts (without a common return plenum) before transport to the main return air duct. Thus, bioaerosols produced in an occupied space may be circulated to other parts of a building, and particles in this air stream may settle on surfaces in the ducts or plenums through which the air travels. Backflow from a return or exhaust air system could re-aerosolize these particles. Water leaks in return air systems may also contribute to microbial contamination in this part of an HVAC system. Thus, backflow of air could also deliver fungal spores to the occupied zone.

4.6 Protecting Investigators and Building Occupants During Walkthrough Inspections

4.6.1 Assessing the Risk of Bioaerosol Exposure

During any building evaluation, investigators must be aware of the potential for exposure to biological agents and the possibility that their activities may increase exposures for building occupants. Actions that may release high concentrations of fungal spores and other biological particles include accessing the interiors of HVAC systems, exposing contamination when removing wall or floor coverings or lifting ceiling tiles, and collecting bulk samples of contaminated building materials. Inspecting and collecting water samples from operating cooling towers in legionellosis investigations may also place investigators at risk of exposure to airborne bacteria. Therefore, systems should be shut down during inspection, if possible. The uncertainties about the risks associated with potential exposure to biological agents during walk-through building inspections are similar to those faced by remedition workers [see 15.2.3.6].

Disturbance of large accumulations of bird, bat, or rodent droppings presents an exposure risk for building inspectors because of the large numbers of fungal spores and hyphal fragments as well as other potentially infectious or allergenic materials and organic dust that may be released. Investigators should use reasonable precautions when entering areas where there may be animal infestations and when examining and removing materials from such areas (Lenhart, 1994; Lynn et al., 1996; Lenhart et al., 1997). Given the difficulties of testing animal droppings for infectious and antigenic biological agents, investigators should assume that exposure to animal droppings and nesting materials in buildings or air-handling systems may be hazardous and should take appropriate precautions [see 15.6].

4.6.2 PPE Selection and Use

While conducting building inspections, investigators may encounter hazardous biological agents as well as chemical and physical hazards. The best protection against exposure is the use of common sense and the anticipation and avoidance of hazardous situations. In addition, investigators should use personal protective equipment (PPE) appropriate for the suspected hazards to which they may be exposed. Such decisions require *a priori* awareness of potentially hazardous agents, significant exposure routes (e.g., inhalation, skin or mucous membrane contact, or ingestion), and probable concentrations of the biological agents

in the materials known to be or suspected of being contaminated [see also 9.2.1, 15.2, and 15.6]. For example, investigators closely inspecting and sampling small, localized patches of fungal growth on a wall may decide they do not need PPE or that respirators and disposable gloves are sufficient. Other situations may warrant the additional use of reusable or disposable coveralls and protective eyewear and footwear. Investigators entering an attic with a large accumulation of bird or bat droppings or extensive fungal growth may need full-face-piece, powered air-purifying respirators; disposable protective clothing with hoods; disposable latex gloves under cotton work gloves; and disposable shoe coverings (Lenhart, 1994).

Airborne fungal spores generally range from 1 to 50 μm, and most other bioaerosols are in a similar size range. Therefore, in many circumstances, a disposable N-95 NIOSH-approved respirator (which removes 95% of particles ≥0.3 μm) offers adequate protection (Chen et al., 1994; Jonson et al., 1994; Willeke et al., 1996; Qian et al., 1997, 1998; Wake et al., 1997). However, the facepiece must fit tightly to ensure that contaminants do not enter through leaks between the respirator and a wearer's face. Respirator use has been recommended to prevent exposure to the agents of legionellosis (CDC, 1997b) and hantavirus pulmonary syndrome (CDC, 1993c). Specific environments may require more protective respirators [see Chapter 15].

Investigators must appreciate what is involved when they decide they need respiratory protection. Respirator use must follow a complete respiratory protection program as specified by the Occupational Safety and Health Administration (OSHA) Standard 29, Code of Federal Regulations 1910.134 (NIOSH, 1996). OSHA requires that respiratory protection programs include written standard operating procedures; respirator selection on the basis of hazard; user instruction and training; respirator cleaning, disinfection, storage, and inspection; surveillance of work area conditions; evaluation of the respirator protection program; medical review; and use of certified respirators.

4.6.3 Protecting Building Occupants During Walkthrough Inspections

Building occupants may be affected by the activities of investigators who are conducting a building inspection if biological agents are disturbed and become airborne. When possible, inspections that may release bioaerosols should be conducted when the study site is unoccupied. If this is not possible, all work should be conducted carefully to minimize the dissemination of contaminants.

Investigators should consider their own health and safety and wear PPE whenever they decide it is necessary. However, they also should consider how building occupants may react to the presence of visitors in protective clothing and respirators. Therefore, building occupancy should be kept to a minimum during inspections for which the investigators have decided that PPE is required. This will prevent accidental exposure of building occupants and avoid raising anxiety among them.

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Appendix 4.A. Building Walkthrough Checklist
(letters in parentheses identify components illustrated in Figure 4.1)

1. Surrounding Area

Type of location (urban, suburban, rural): _____

Surrounding land use (business, residential, agricultural): _____

Animal-confinement operations?	Yes	No
Construction or agricultural activity?	Yes	No
Water sprays (e.g., fountains, irrigation)?	Yes	No
Cooling towers present?	Yes	No
Shallow groundwater areas (e.g., marshes, bogs)?	Yes	No
Site drainage adequate?	Yes	No
Vegetation surrounding building?	Yes	No

2. Heating, Ventilating, and Air-Conditioning System

a. *General Characteristics*

Type of ventilation system: _____

Location of air-handling units: _____

Cooling method used: _____

Heating method used: _____

Locations served by individual air handlers: _____

b. *Outdoor Air Intake*

Location: _____

Bird screen present?	Yes	No
Feathers or bird droppings near or in OAI?	Yes	No
Other organic matter near or in OAI (e.g., leaves, plant down, insects)	Yes	No
OAI protected from rain, snow, fog?	Yes	No
Standing water or evidence of standing water near or in OAI?	Yes	No
Cooling tower within 7.5 m (25 ft)?	Yes	No
Exhaust air outlet within 7.5 m (25 ft)?	Yes	No

c. *Filters* (A)

Filters present and free of organic debris and microbial growth?	Yes	No

d. *Mixing Chamber of Air-Handling Unit*

Mixing area clean and free of debris and microbial growth?	Yes	No
Malodors?	Yes	No
Evidence of water damage or intrusion?	Yes	No

e. *Heating and Cooling Coil Area* (B and C)

Coils clean and free of organic material and microbial growth?	Yes	No
Condensate pan and drain present?	Yes	No
Condensate pan well drained (i.e., no standing water, biofilm, or residue)?	Yes	No
Corrosion on pan?	Yes	No
Malodors?	Yes	No
Evidence of water transport from coil area to other areas?	Yes	No

f. *Spray Humidifiers, Evaporative Coolers, or Air Washers* (D)

Type of unit: _____

Chemicals or additives used: _____

Maintenance schedule: _____

Type of medium, if any: _____

Microbiological samples of the water taken routinely?	Yes	No

If yes, results: _____

Recirculated water used? ..... Yes No
Biofilm, dirt, or microbial growth in sump area? ..... Yes No
Malodors? ..... Yes No
Water leakage from humidifier into duct system? ..... Yes No
Water pooled near unit? ..... Yes No
Unit enters airspace directly (or ducted to other areas)? ..... Yes No

g. ***Supply Side of Air-Handling Unit*** (E, F, and G)
Where do ducts enter building (e.g., at ceilings, below floors): _____
Type of supply ducts (lined or unlined): _____
Supply area clean and free of debris and microbial growth? ..... Yes No
Malodors? ..... Yes No
Evidence of water damage or intrusion? ..... Yes No

h. ***Return Side of Air-Handling Unit*** (H, I, J, and K)
Type of return (ducted or plenum): _____
Porous lining on ducts or plenums? ..... Yes No
Return area clean and free of debris and microbial growth? ..... Yes No
Malodors? ..... Yes No
Evidence of water damage or intrusion? ..... Yes No

3. **Building/Occupied Space**
Number of floors: _____
General building uses: _____
Attic present? ..... Yes No
If yes, condition: _____
Basement or crawlspace present? ..... Yes No
If yes, condition: _____
Water features (e.g., fountains, sprays, indoor waterfalls)? ..... Yes No
Malodors? ..... Yes No
Visible microbial growth? ..... Yes No
History of water damage? ..... Yes No
Evidence of water damage (stained or discolored ceiling tiles, walls, floors, carpeting)? ..... Yes No
Condensation on walls and windows? ..... Yes No
Window air conditioners? ..... Yes No
Evaporative air coolers? ..... Yes No
Sump pump used? ..... Yes No
Fancoil and induction units? ..... Yes No
Potted plants? ..... Yes No
Portable air cleaners? ..... Yes No
If yes, why? _____
Console humidifiers? ..... Yes No
If yes, why? _____
Console dehumidifiers? ..... Yes No
If yes, why? _____
Typical RH levels in the building: _____

Chapter 5

Developing A Sampling Plan

5.1 Introduction

5.1.1 The Sampling Plan

Often the hypotheses investigators develop to explain possible bioaerosol exposures can be tested by collecting environmental samples. The hypotheses explain *why* the investigators have decided to sample a work environment for biological agents and also describe *what* agents they intend to study. A sampling plan describes *where*, *when*, and *how* investigators propose to collect samples to test their hypotheses. The following discussion outlines a rigorous and scientifically sound approach to studying biological agents in indoor environments. Unfortunately, investigators often must reach a compromise between an ideal sampling plan and one that is feasible. Constraints on sampling may involve the time, manpower, instruments, analytical resources, and the access to the building. Each investigation will have its own practical constraints, and investigators must decide case by case how to reach their goals without jeopardizing the credibility, representativeness, and integrity of the data they collect.

Table 5.1 outlines typical plans to sample four biological agents. In this table, sample analysis is intentionally placed before (to the left of) sample collection to remind investigators that these steps are coupled. Once an investigation team has decided what biological agents to study, the investigators should contact a laboratory and discuss sample analysis before collecting samples. Readers can also follow this table from right to left, studying first the column marked Sampling Plan and then reading the columns Sample Collection and Sample Analysis to see how a plan can be executed.

A sampling plan includes specification of (a) the biological agents under study, (b) typical sources and reservoirs of the suspected agents, (c) the anticipated concentration and variability in space and time for each agent, (d) the analytical methods that will be used to detect, identify, and quantify the agents, (e) the sampling methods to be used, (f) the operating parameters of all sampling instruments, and (g) the locations and times at which samples will be collected.

Table 5.2 outlines the primary steps in the process of collecting environmental samples to test hypotheses.

5.1.2 Environmental Sampling

Investigators may decide to sample for biological agents in indoor and outdoor environments for a variety of reasons. Among these purposes are determining what kinds and relative concentrations of biological agents are present for comparison with another location. Investigations may also be undertaken to monitor changes that may occur over time and to detect existing or emerging problems. Sampling for specific biological agents may be undertaken to locate their sources as well as to esti-

mate exposures or document that exposure may occur. If problems are found, sampling can help investigators develop and implement control strategies and assess their effectiveness. Sampling results may be used to determine what degree of PPE is needed during a building inspection or a remediation operation. Occasionally, environmental sampling is undertaken in response to an accident or emergency to evaluate possible consequences of the unusual event. Some investigations are conducted as research efforts to assess or validate sampling methods or to better understand environmental conditions and occupational exposures.

The word "sample" means different things in different contexts. At times, investigators use the term to designate an individual measurement (e.g., an air or source sample). However, a sample may also designate a set of measurements (e.g., multiple measurements of some parameter that comprise a sample of size *n*). In either case, the goal of sampling is to learn about entire populations by looking at subsets of the members of the population. For example, investigators may attempt to characterize exposure conditions in a building by measuring concentrations of environmental contaminants at selected places and times chosen to be as similar as possible to where and when workers may be exposed. The key to accomplishing this goal is to select samples so that they (a) accurately and completely represent the entire population, or (b) represent the conditions most likely to be associated with adverse effects.

TABLE 5.1. Example: Bioaerosol Sampling Plans

Agent	Sources	Sample Analysis	Sample Collection	Sampling Plan
Fungal allergens	Outdoor air, Fungal growth in HVAC system	Culture • Identify major taxa Immunoassay • Assay for specific antigens Microscopy • Identify major taxa	Bulk samples • Pieces of material Surface samples • Adhesive tape or swab samples	Collect bulk and surface samples from materials with suspected fungal growth
		Culture • Count colonies and identify major taxa Immunoassay • Assay for specific antigens Microscopy • Count spores and identify major taxa	Air samples • Agar impactor • Filter cassette • Spore trap	Collect air samples outdoors, near suspected contamination, and in the occupied space with the HVAC system off then on
Legionella sp.	Cooling tower, Hot water supply	Culture Direct fluorescent antibody stain • Count colonies or cells	Bulk samples • Water, biofilm (Air samples not recommended)	Collect water from cooling tower sump, hot water tank, and municipal water supply
Endotoxin	Humidifier	Limulus amebocyte lysate assay • Assess concentrations of endotoxin	Bulk samples • Water	Collect water from reservoir before and after cleaning
			Air samples • Filter cassette	Collect air samples with humidifier off then on before and after cleaning water reservoir
Stachybotrys toxins	Wet wallboard, *Stachybotrys chartarum*	GC-MS toxin analysis • Macrocyclic trichothecenes	Bulk samples • Pieces of material	Collect bulk and surface samples from materials with suspected funal growth
		Microscopy • Identify spores • Identify and count *Stachybotrys chartarum* spores	Surface samples • Adhesive tape Air samples • Spore trap	Collect air samples while area is unoccupied then during normal use

As much as possible, the relative proportion or concentration of a biological agent in a sample should reflect that in the original material. To achieve this goal, a sample must be handled so that it does not deteriorate or become contaminated before analysis (i.e., biological agents that are present should not be lost and agents that are not present in the original sample should not be introduced). Viable organisms in samples should change as little as possible after removal from a test environment (i.e., they should not multiply appreciably nor lose culturability). Table 5.3 outlines points to consider in designing a sampling plan.

Environmental sampling may provide clear evidence supporting a hypothesis that investigators have formulated. For example, for the first situation in Table 5.1, the bulk samples may show high concentrations of a particular fungus and the air samples may show a higher concentration of the same fungus when the HVAC system is operating than when it has been off overnight and the building has been unoccupied. These findings may convince the investigators that the fungus is growing within and disseminated by the HVAC system. Conversely, negative results may persuade investigators to abandon this hypothesis and to construct others. For the second situation, failure to identify *Legionella* spp. in the water systems of a building may suggest that exposure for a patient with Legionnaires' disease may not have occurred at the workplace and that the investigator should look elsewhere for the exposure source to prevent other infections.

Investigators are cautioned that sampling results are not always easy to interpret and that incomplete bioaerosol data may confuse and complicate an investigation rather than provide clarity. For example, the types and relative proportions of bioaerosols in indoor and outdoor air are highly variable, occasionally making it difficult to see differences between test and control sampling sites. Also, many microorganisms to which everyone is exposed throughout life are opportunistic pathogens. A laboratory report that indicates that many of the microorganisms isolated from a workplace may cause disease (even if this is unlikely in healthy persons) can cause unnecessary alarm and require lengthy explanation. Therefore, study design is often the single most important element of an investigation. Even the most careful and sophisticated sample collection and data analysis will not salvage a poor study design. Investigators must define their hypotheses as clearly as possible at the beginning of a study and determine what kinds of data they need and what information they can expect to obtain with a proposed sampling plan.

The following discussion focuses on air sampling for biological agents, the intention of which is usually to identify what agents are present and to estimate concentrations quantitatively or semi-quantitatively. Investigators should not underestimate the potential value of source samples even though this chapter does not discuss bulk and surface sampling as extensively as air sampling. All bioaerosols have a source, the identification of which is often key to eliminating or minimizing the presence of a biological agent in indoor air.

Tracing the origin of industrial pollutants in a workplace is often fairly straight forward because the materials used in a manufacturing process are generally the sources of the compounds to which workers are exposed. Therefore, it may be known that a particular, potentially hazardous material will be present in a workplace atmosphere. In such case, the purpose of sampling would be to determine the air concentration of that material. However, the biological agents of potential interest in BRIs are usually not part of a manufacturing process but environmental contaminants for which there may be more than one origin. Identifying which single or multiple biological or nonbiological agents are responsible for workers' complaints and identifying sources to allow control is often a challenging undertaking.

5.2 Sample Collection

When developing sampling strategies, IHs, EHPs, and IEQ consultants must consider possible sources of error, identify the desired precision and accuracy of the measurements to be made, and specify the degree of confi-

TABLE 5.2. Fundamental Steps When Collecting Environmental Samples to Test Hypotheses

1. State the objective of the study and outline the goals and expectations of the sampling program.
2. State the explicit questions to be addressed and the information needed to answer them.
3. Design a sample collection plan to obtain the required information.
4. Collect and analyze samples.
5. Compile and summarize data.
6. Perform statistical tests, if appropriate.
7. Determine if the objectives of the sampling program have been met and if the questions posed at the outset of the investigation can be answered.

TABLE 5.3. Points to Consider When Designing a Sampling Plan

- The nature and expected concentrations of biological materials.
- The relevance of average or worst-case concentration measurements.
- The cost and availability of various types of sample analyses.
- Constraints that analytical methods may impose on sample collection.
- The suitability, cost, and availability of sample collection devices and related supplies.
- Constraints the time and method of sample transport to an analytical laboratory may impose.
- The technical expertise required of field and laboratory personnel.

dence they need to interpret the results (Gross and Morse, 1996). These decisions are coupled with the practical questions of *where*, *when*, and *how* to measure biological agents as well as *how many* samples to collect, as discussed in this section. Investigators base their decisions on their training, experience, and the approaches other investigators have used successfully in similar settings.

5.2.1 Where and When to Sample

5.2.1.1 Typical and Worst-Case Exposure Assessments

Workplace investigations are driven by the need either to (a) establish typical worker exposures and exposure conditions, or (b) measure worst-case exposures. For comparison, background, baseline, or best-case measurements may be collected at outdoor or control locations or during non-work periods. Direct-reading instruments would make identification of ideal sampling sites and times easier. However, although a few such devices exist, they are not widely available to measure occupational bioaerosol exposures. Therefore, in deciding where and when to collect appropriate bioaerosol samples, investigators must learn to combine information they gathered from site visits with information they have acquired from interviews and literature reviews. A site visit provides information on specific worker activities and work processes. Background knowledge, prior experience, and discussions with workers and managers provide information on potential agents, their sources, and factors that may lead to exposure. Many investigations begin only after workers have developed BRSs or a BRI, at which time exposures may no longer be occurring or occurring at the original level. Therefore, it may be necessary to try to recreate environmental conditions that existed previously.

Personal samples are the best way to estimate inhalation exposures. However, few personal samplers are available for collecting biological agents. Therefore, samples collected in a worker's breathing-zone are often used to estimate inhalation exposures. Area (or static) samples may be adequate to estimate average bioaerosol concentrations. For example, air samplers may be placed at the desks of stationary workers or located centrally in an area that several mobile workers share. To establish typical exposures that represent an entire population, the samples should represent the entire range of exposures and conditions. One way to achieve this is to select sampling sites and times randomly (i.e., all persons and work periods should have an equal chance of being monitored) (Appendix 5.A).

To estimate maximum or worst-case exposures, samples are collected near suspected bioaerosol sources, symptomatic workers, or the workers assumed to have the highest exposures. Sampling to measure maximum or worst-case exposures could be considered a form of stratified sampling, where the strata are defined according to the investigators' expectations. A stratified sampling plan can also be used to compare exposures at two or more sites. In such cases, the investigators decide in advance to sample subgroups of the population separately. Within the zones of anticipated high and low exposures, the sites or workers to be monitored can be chosen using a random selection process so that all areas are adequately represented (Appendix 5.A). Stratified sampling is also used when particular subgroups of interest are small and the investigators want to ensure that a study includes at least some members from each subgroup. For example, investigators may suspect that exposures for a few part-time workers are different than for full-time workers. However, given the small number of part-time workers, few or none of them may be included in a study if participants were chosen strictly randomly. Therefore, investigators may choose initially to study the two groups separately, collecting equal numbers of samples to represent exposures for the two populations. If exposures are determined to be the same, the data for the part-time workers could be combined with that for the other workers.

Sampling goals need to be clearly defined to develop a sampling plan, and the sampling plan should be clearly developed before samples are collected. Some measurements of worst-case exposures might correctly be included in a random or representative sample. However, it is difficult, if not impossible, to estimate average exposures from samples intentionally chosen to represent worst-case conditions. Table 5.4 summarizes the decisions involved in determining where to collect bioaerosol samples. The steps outlined in this table apply to bioaerosol sampling in research studies as well as problem-building investigations.

5.2.1.2 Averaging Times for Bioaerosol Sampling

Occupational exposure limits are often established as allowable air concentrations for averaging times appropriate to particular health hazards. An averaging time is the period over which an air concentration (exposure) is measured. A time-weighted average concentration can be calculated when concentrations and their time durations are known. Typical averaging times are (a) a conventional 8-hour work shift and a 40-hour work week, or (b) a 15-min., short-term exposure period. The appropriate averaging time for occupational exposure assessment depends on whether health effects are anticipated to be cumulative or acute. Cumulative health effects are those that result from repeated (chronic) exposures (e.g., lead poisoning), whereas acute health effects arise from single, sufficiently high exposures (e.g., carbon monox-

ide poisoning). Monitoring for chronic hazards is usually conducted for the duration of one or more work shifts (Jensen and O'Brien, 1993). Establishing a mean air concentration, with appropriate confidence limits, may provide a good basis for estimating exposures associated with long-term or chronic health risks (Rock, 1995). On the other hand, an upper confidence limit or 95th percentile of an exposure distribution may more accurately indicate a worker's acute health risk (i.e., that associated with infrequent but high exposures). Section 5.2.4 discusses the minimum numbers of samples recommended for estimating confidence intervals and sample variance.

Analogous averaging times have not been established for chronic and acute bioaerosol exposures, except for those biological agents listed in the TLV booklet (see 1.2). Some biological agents can be considered to pose both chronic and acute risks. For example, repeated exposure to an antigen may lead to the development of HP in previously unsensitized workers whereas a single, acute exposure could cause immediate allergic reactions in already sensitized workers. Mycotoxins are another group of biological agents known to manifest different effects for chronic and acute exposures, at least by ingestion. To study long-term or chronic health risks, continuous spore-trap samplers for microscopic examination; 8-hour, filter-cassette samples for allergen assay; or multiple, discrete, agar-impaction samples for culture of viable microorganisms may be used to assess worker exposure. For example, Robins et al. (1997) used sequential sampling with filter-cassettes to characterize the health effects of chronic exposures to bacterial aerosols (including endotoxins). To study acute exposures to biological agents, investigators would want to characterize peak, short-term exposures, perhaps while maximizing activity in the space. For example, worst-case sampling might help identify what allergen exposures provoke acute asthma attacks in sensitized workers.

Mechanical disturbances to potential sources of biological agents and human activity during sample collection may affect the type and amount of material in the air. Therefore, during sample collection, the operation of ventilation system equipment and the activities of occupants should reflect typical patterns of building use. Exceptions would be monitoring of actual or simulated worst-case conditions during which equipment operation may be modified and occupant activity may be maximized. Simulating worst-case circumstances has been termed "aggressive sampling," which should be consistent with what might actually occur in an occupied, operating building (see 14.2.4.1). Table 5.5 summarizes the types of samples discussed above and gives examples of time patterns for collecting air samples.

5.2.1.3 Practical Considerations For many investigations, resources are a major limiting factor and determine what type of study can be conducted. Investigators must decide how best to use available field personnel, sampling equipment, and laboratory services to meet a client's needs. A clear understanding of the questions at issue and the information a client expects to receive can help an investigator decide how to design a study. The services an investigator will provide and the scope of the responsibilities an investigator will undertake should be defined clearly in a written contract, memoran-dum or letter, or other applicable type of signed agreement.

When deciding where, when, how, and how many samples to collect, investigators must consider the number of sampling devices available for a study. If mul-

TABLE 5.4. Deciding Where to Collect Bioaerosol Samples to Compare Anticipated High and Low Exposures

- Tentatively identify bioaerosol sources (e.g., HVAC system components, building materials, or furnishings that are visibly contaminated with microbial growth or that show signs of water damage) and estimate their source strengths (i.e., bioaerosol generation rates). Predict spatial and temporal bioaerosol concentration gradients.
- Identify zones with expected differences in bioaerosol kind or concentration (e.g., indoor and outdoor sites as well as areas near suspected sources and control areas).
- Identify occupants anticipated to receive the highest and lowest exposures and show the strongest and weakest reactions based on their proximity to potential sources, types of activities conducted, and medical condition.
- Identify areas to which investigators will be allowed access. Identify areas that can be monitored without disrupting typical occupant activities.
- Select at least one, preferably three, sampling locations in each of the following areas
  - An anticipated high-exposure area
  - An anticipated low-exposure area
  - Outdoors near air intakes for the building.

  If applicable, also sample at the following locations
  - Outdoors near potential sources of bioaerosols that may enter a building
  - Outdoors high above grade and away from potential bioaerosol sources.

tiple, comparable instruments are available, they can remain at different sampling locations throughout a study. Otherwise, investigators need to arrange a rotation system to monitor all selected sampling sites in a way that does not compromise the study design. For example, it may not be correct to compare test and control samples if they were collected at markedly different times of day due to the time required to disassemble, move, and reassemble the sampling equipment. Therefore, equipment and field personnel need to minimize the time between collection of samples at sites that will be compared, or the investigators should collect a sufficient number of samples at each site to characterize the different locations well. Randomization of sample sequence can help minimize bias [see Appendix 5.A]. Investigators should also determine if their presence and activities may change the way work is conducted and, if so, how these changes may affect bioaerosol exposures.

5.2.1.4 Example of Where and When to Sample Air samples are typically collected outdoors and indoors, both before and after potential sources of microbial growth are disturbed. Outdoor samples are usually collected at the highest point of a building (e.g., the rooftop) facing into the wind as well as near building air intakes. For naturally ventilated buildings, reference samples should be collected where they will sample outdoor air representative of the air that may enter buildings through open windows and doors. Outdoor samples should be collected throughout the period of indoor sampling (Table 5.4). Table 5.6 outlines an example sampling plan designed to assess the contribution to the indoor atmosphere of fungi growing in ventilation ducts.

5.2.2 Choosing an Air Sampler

An investigator selects one or more sample collection methods and gathers samples based on (a) the agents of interest, (b) the analytical methods by which the laboratory will identify and perhaps quantify the biological agents or indicators of the active agents, and (c) the sites and times at which samples will be collected. Chapter 2 describes how investigators identify the biological agents they wish to collect by combining information gathered during preliminary interviews and site inspections. The locations and time patterns chosen for sample collection (Tables 5.4 and 5.5) depend on what information the investigators need to obtain to evaluate the hypotheses they have formed about the setting.

Chapter 11 describes bioaerosol samplers, their operation principles, applications, and limitations. Investigators often adopt the same collection device others have used for a particular biological agent in a similar setting. Familiarity with recent published literature describing collection devices and study designs can help investigators in the sampler-selection process (Burge, 1995; Cox and Wathes, 1995; Health Canada, 1995; AIHA, 1996; ISIAQ, 1996; ASM, 1997). Table 11.1 summarizes commercially available samplers and their applications. Table 5.7 outlines a process for selecting an air sampler to collect bioaerosols.

5.2.3 Sample Volume and Sample Collection Time

Sample volume and collection time are related through the minimum air volume needed to detect a target material present at a given concentration. In turn, sample volume and collection time depend on the volumetric flowrate of a bioaerosol sampler. The *lower detection limit* (LDL) for a bioaerosol sampling method can be deter-

TABLE 5.5. Time-Related Patterns for Collecting Bioaerosol Samples

CAVEAT: Stated sampling methods and sample collection times are given for illustration only.

Time Pattern	Description and Example (samples collected at least in duplicate)
Continuous	Sample continuously or in series throughout the time interval of interest, for example, (a) a continuous, 8-hour, filter sample, or (b) eight, sequential, 1-hour samples with a moving-slide, slit sampler.
Periodic	Divide the total time period into equal segments and collect samples during the same portion of each segment, for example, (a) 1-min., direct-agar impaction samples during the last 10 min. of every hour for 8 hour, or (b) 10-min., portable spore-trap samples at 10 a.m. and 2 p.m. each day for three, consecutive days.
Random	Use a random selection process (Appendix 5.A) to choose sampling periods from all intervals in a chosen averaging time, for example, 5-min., direct-agar impactor samples during eight, randomly selected, 5-min intervals in an 8-hour work day.
Worst-case	Collect samples before, during, and after activities known or expected to create worst-case exposures, for example, 15-min., liquid impinger samples (a) before disturbance begins, (b) during peak activity, and (c) after the effects of the disturbance have subsided.

mined from (a) the minimum amount of material that an assay can detect, (b) the recovery efficiency for the assay method and any concentration steps that may apply, (c) an air sampler's flow rate, and (d) the maximum sample collection time. The minimum detectable amount of a biological agent may be 1 CFU for a culturable microorganism on an agar plate, 1 fungal spore per microscope field for a spore-trap sample, or a minimal mass amount of allergen per milliliter of a dust extract. Laboratory personnel may use blank samples to determine the background amount of test material that may be present on unexposed collection media or in storage containers. *Upper detection limits* (UDLs) can be determined similarly, taking into account minimum sample collection times and any dilution steps that may apply (see 5.2.3.1 and 5.2.3.2).

Investigators aim for sampling times that are (a) sufficiently long to collect a detectable and representative numbers of particles or other amount of material (Nevalainen et al., 1992, 1993), but (b) short enough to avoid masking (i.e., the overlap of particles on microscope specimens or the contact of colonies on culture media) (Chang et al., 1994, 1995). Laboratories should report the UDL and LDL of a method along with sample results. In particular, LDLs should be specified when a target biological agent is not found because all that can be concluded is that the agent was not present at or above the LDL. Table 11.1 lists the airflow rates for several bioaerosol samplers and Table 11.5 identifies approximate air concentrations for which these samplers are suitable. The American Industrial Hygiene Association (AIHA) *Field Guide* also provides ranges and sensitivities for commonly used bioaerosol samplers (AIHA, 1996).

Short- and long-term variations in bioaerosol concentration along with typically short collection times dictate collection—at a minimum—of sequential or simultaneous replicate samples to obtain confident estimates of bioaerosol concentrations. Where feasible, longer sample collection times may reduce the variability seen with shorter sampling times. For example, Stanevich and Petersen (1990) found that increasing sample collection time from 1 to 5 min. (at 28 L/min) reduced intra-sample variability. Therefore, sampling at a lower airflow rate for a longer time is preferable to sampling at a higher rate for a shorter time, provided the longer sampling time does not adversely affect recovery of the biological agent of interest. Collection of sequential, short-term samples can provide information on concentration fluctuations while also providing an estimate of the long-term average.

5.2.3.1 Direct-Agar Impaction The density of colonies growing on a culture plate affects the reliability of the information that can be obtained. Too few CFUs inaccurately reflect what is present, whereas too many CFUs are difficult to count and examine. Therefore, when using direct-agar impactors, it is especially important to anticipate bioaerosol concentration correctly and to collect samples that will yield an appropriate colony surface density. In general, 25 to 250 bacterial colonies and 10 to 60 fungal colonies are considered optimal for accurate counting and identification of CFUs on standard, 100-mm plates. Target colony counts are adjusted proportionally for smaller and larger plates. Therefore, another rule of thumb microbiologists have used is a maximum surface density of 1 colony/cm^2. For multiple-hole impactors, investigators should also consider the greater

TABLE 5.6. Example: Sampling Plan to Assess the Contribution of Fungal Growth in a Ventilation System to the Presence of Fungal Spores in Indoor Air

(Repeat series at least twice on two or more days; collect at least duplicate samples at all sites)

Outdoors	• Site remote from obvious bioaerosol sources: initial background or baseline (initial) measurement of highest quality general air • Site near outdoor air intake: initial background or baseline measurement of air entering the building
Indoors	• Site near a supply air diffuser: air delivered from ventilation system, as follows: – When the air distribution system has been turned off at least overnight (preferably for a weekend) – Immediately after the air distribution system is restarted, with the sampler oriented so that air from the diffuser directly enters the sampler – After the air distribution system has been operating for 30 min. – During gentle or vigorous mechanical agitation of the ductwork, as appropriate (Caution: aggressive sampling should only be conducted when a space is unoccupied and should be consistent with what might occur during normal building operation and use) • At least three locations in the space served by the supply air diffuser (sampler at occupant breathing height): occupant exposure
Outdoors	• Site remote from obvious bioaerosol sources: final background or baseline (final) measurement of highest quality general air (see above) • Site near outdoor air intake: final background or baseline measurement of air entering the building (see above)

variability associated with higher plate counts when using a positive-hole correction [see 11.5.1.1].

Table 5.8 identifies the maximum and minimum air concentrations for three samplers based on an optimal density of fungal colonies and commonly used sample collection times. Obviously, it is possible to count a single colony on a culture plate. However, until colony counts reach ~10, variability between simultaneous samples is very large, and samples with so few CFUs should be avoided. Therefore, 10 CFU/plate is listed as the LDL in Table 5.8. Investigators can perform similar calculations for other air samplers. This table also illustrates how, for a given collection time, investigators can identify a suitable sampler (based on airflow rate) for an anticipated air concentration. For example, compare the concentration ranges suitable for the two multiple-hole impactors for a 1-min sampling time.

Knowing the air flow rate and plate size of an available device, an investigator would determine how long to sample by anticipating in what range the air concentration is likely to fall. For example, consider an anticipated air concentration of approximately 100 CFU/m³. An investigator could operate the 90-L/min multiple-hole impactor for 1 min, the 28-L/min multiple-hole impactor for 10 min, and the 50-L/min slit-to-agar impactor for 15 min. Similarly, for an air concentration of approximately 1000 CFU/m³, the 28-L/min and 50-L/min impactors could be run for 1 min. However, the 90-L/min sampler would not be appropriate in this environment because of its high flow rate and limited agar surface (i.e., the resulting colony density would exceed the recommended limit). A sampling time sufficiently short to avoid overloading the agar surface would be too short to be practical. For unknown air concentrations, multiple sampling times are needed. For example, an investigator could collect samples for 0.5, 3, and 10 min. with the first multiple-hole impactor to cover the respective concentration ranges of approximately 35-275, 120-1000, and 700-5500 CFU/m³.

5.2.3.2 Spore-Trap Samples Considerations similar to those discussed in Section 5.2.3.1 for direct-agar impactors apply to particle counting by microscope. Investigators should consult a knowledgeable laboratory analyst to learn the collection surface area, ideal particle surface density, and fraction of the total collection surface that is typically examined. Knowing the airflow rate and estimating the air concentration, the investigators can determine appropriate sample collection times. A potential problem with microscopic analysis is that non-biological particles can be as or more abundant than bioaerosols, causing masking. If suspected, such interference can be estimated by conducting a pilot study. Investigators can also collect preliminary samples to estimate air concentration before conducting more detailed sample collection.

5.2.3.3 Calculating Optimal Sampling Time Nevalainen et al. (1993) discuss calculation of optimal sampling time for bioaerosol samplers. Equation 5-1 gives a method to calculate optimal sampling time, t, when the desired surface density, δ, deposit area, A, average air concentration, C_a, and airflow rate, Q, are known:

TABLE 5.7. Selecting an Air Sampler for Bioaerosol Collection

Determine what specific biological agents may be present in a test environment and which of these are of interest for the current investigation. Determine what indicators will be used to measure the biological agents of interest, and confirm that the laboratory can analyze samples to identify and quantify these agents or indicators.

Determine what information is needed about the targeted biological agents or indicators.

- Concentration (e.g., colony-forming units (CFU)/m³, number/m³, or mass/m³).
- Identification of specific microorganisms (e.g., presence/absence) or identification of all isolates and their relative concentrations.
- Average air concentration, worst-case concentration, or personal-exposure assessment.
- Particle size distribution; separate collection of respirable/non-respirable particle fractions.

Visit each test site and identify possible constraints on bioaerosol sample collection.

- High bioaerosol concentrations - collection surfaces may be overloaded unless the airflow rate is sufficiently low or the collection time is sufficiently short.
- Low bioaerosol concentrations - insufficient sample material may be collected unless airflow rate is sufficiently high or the collection time is sufficiently long.
- Temperature extremes - only short collection times may be possible for direct-agar impaction and liquid impingement in hot, dry areas or where collection media may freeze (e.g., outdoors in summer or winter, respectively).
- Collection from a moving air stream where inlet velocity and orientation will be a concern (e.g., within ventilation ducts, outdoors, at supply air diffusers).
- Access to electrical power.
- Need to collect samples without disturbing occupants or interfering with their activities.

Review literature to learn what devices other investigators have used satisfactorily to collect the target biological agent or indicator in similar work environments.

$$t = \frac{\delta A}{C_s Q} \qquad (5\text{-}1)$$

Sampling time is typically calculated in minutes, surface density in CFU or number of particles per unit of collection surface area (e.g., 1 CFU/cm² on an agar surface or 10^4 particles/cm² on a surface to be examined by microscope), deposit area in cm², air concentration in CFU/m³ or particles/m³, and airflow rate in L/min or m³/min.

5.2.4 Sample Number

Factors that investigators consider when deciding how many samples to collect include (a) the objective of sample collection, (b) the spatial and temporal variability of the parameter being measured, (c) equipment limitations, and (d) manpower limitations. The number of samples investigators should collect depends on how they intend to use the data. Other things being equal, the larger the sample size the better (i.e., measurements and differences between sets of measurements are more precise for greater sample sizes). Here sample "size" means the number of measurements or samples collected, not the mass or volume of a sample—although this may also be important in determining the representativeness of a source sample and providing sufficient material for laboratory testing. Statisticians can help investigators calculate the number of samples needed to discern a difference of a given magnitude with a chosen probability of detection at a specified level of statistical significance. Smaller differences, higher probabilities of detection, and higher levels of statistical significance require larger sample sizes. For a given sample size, a significant difference may only be detectable if the difference is large, a low probability of detection is acceptable, or a low degree of significance is convincing.

For example, consider a biological agent whose mean concentration is 100 with a standard deviation of 25 in some arbitrary units. Investigators are interested in differences they estimate may be on the order of 30 between this sampling site and another (i.e., the second site could be ≤70 or ≥130). The investigators choose a high significance level ($\alpha = 0.05$, that is, only once in 20 times would they declare a difference significant when it is not—a false-positive result). To see a difference under these conditions, the investigators would need to collect 5 to 6 samples. Two to three samples would suffice if the investigators were willing to accept a higher false-positive rate (e.g., 0.20 rather than 0.05, that is, they would falsely declare that a difference is significant four times as often as before or approximately once in every five tests). However, to see a smaller difference (±10 instead of ±30) under the original conditions, approximately 50 samples would be needed.

Besides incorrectly declaring that a difference is significant when it is not, investigators can also fail to observe a difference when there is one (a false-negative result). For a given sample size, significance level, sample mean, and sample variance, a statistician can calculate the power of a test. Alternatively, an investigator can decide what probability of detecting a significant difference they wish to have (typically ≥80%; with a 20% chance of missing a real difference) and determine how many samples they would need to collect to achieve this. Standard statistics texts can explain how to calculate sample size and power. Investigators generally assume that important differences will be large and that they will be able to detect them with few samples. However, investigators should keep in mind that with a small sample there often is a fairly high likelihood of missing a real difference.

TABLE 5.8. Calculated Air Concentration Ranges Suitable for Direct-Agar Impactors and Fungal Culture for Selected Sample Collection Times

Sampler	Plate Area (cm²)	Airflow Rate (L/min)	Collection Time (min)	Volume Collected (m³)	Air Concentration (CFU/m³) LDL (10 CFU/plate)	Air Concentration (CFU/m³) UDL (1 CFU/cm²)
Multiple-hole impactor	78	28	0.5	0.014	710	5570
			1.0	0.028	360	2790
			5.0	0.142	70	549
			10.0	0.283	35	276
Multiple-hole impactor	17	90	0.33	0.030	330	567
			0.66	0.059	170	288
			1.0	0.090	110	189
Slit-to-agar impactor	177	50	1.0	0.050	200	3540
			15.0	0.750	10	236
			60.0	3.0	3	59

Unfortunately, investigators seldom have prior data to use to calculate sample size, although a few preliminary samples may suffice to provide an estimate. Leidel et al. (1977) recommended that exposures to substances that have ceiling limits be monitored by sampling during 3, nonrandom, worst-case exposure periods. Rock (1995) has written that, for general occupational exposure assessments, a minimum of 6 samples is required to obtain a valid assessment of the confidence interval around a mean, and a minimum of 11 samples is needed to estimate the variance of a data set. Another recommended approach to characterize worker exposures is to collect samples 3 times a day for 3 consecutive, representative days (Table 5.9). To characterize exposures completely, investigators may need to repeat such sampling seasonally as well as during different modes of building operation and different patterns of worker activity.

In this discussion, it is assumed that all samples will be within a useful concentration range. If there is doubt what that range may be, investigators should collect the recommended number of samples for each air volume they test to cover the anticipated range of air concentration. To whatever number of samples investigators determine they need, they must add a sufficient number of blank or control samples to establish the quality of the data they collect. For example, investigators should collect at least one field blank for every type of sample collected, every different area studied, and every new batch of collection medium, such as filters or culture media (see 13.4). The early inclusion of an epidemiologist or statistician on a project can help investigators avoid errors in study design and data interpretation. While consultation with an epi-demiologist or statistician is not always possible, the references in Chapter 13 and standard texts on epidemiological and statistical methods can guide investigators.

Lest there be any doubt, when speaking of "sample number," what is meant is the number of samples for each type of measurement, not the number of different types of samples to be collected. Therefore, direct-agar and spore-trap samples (respectively for fungal culture and microscopic spore examination) are two separate measurements, each of which requires an appropriate number of samples for each environment to be tested. Investigators must collect multiple samples to characterize exposures at individual test sites. Investigators must collect multiple samples from multiple sites to characterize exposures within a building.

Sampling preceding remediation efforts requires a sufficient number of samples to determine the extent of the area requiring cleaning. Sampling following clean-up requires a sufficient number of samples to demonstrate that the remediation process accomplished its goals. Sampling to demonstrate or rule out exposure to a biological agent is a difficult undertaking and may require the greatest number of samples and a rigorous sampling plan. While a single sample may establish the presence of a biological agent, sampling to demonstrate absence usually requires extensive testing. Therefore, a well-designed sampling plan is particularly important in attempts to demonstrate the absence of a biological agent. Similarly, sampling to convincingly rule out unusual or potentially harmful conditions may be more difficult than demonstrating their presence when they exist.

5.3 Record Keeping

Written procedures for sample collection and analysis ensure consistency from study to study and among field and laboratory personnel over time. Clear and complete records of how an investigation was conducted, by whom, and when; how field personnel collected environmental samples; how laboratory staff analyzed samples; and clear summaries and interpretations of test results are necessary to compile accurate reports, prepare publications, and respond to possible litigation.

Investigators should assign unique identifiers to samples and use these identification numbers throughout sample processing and data reporting. Samples submitted for analysis should be accompanied by a form detailing the numbers, sources, and types of samples collected along with the analyses requested (Table 5.10). Sample forms should provide space for chain-of-custody recordkeeping to track samples from the time of collec-

TABLE 5.9. Suggested Sample Numbers

Purpose	Suggestion (for each sampling site and each sample type)
To estimate worst-case inhalation exposures	Monitor ≥3, non-random, worst-case exposure periods; collect at least duplicate samples for all analyses
To estimate average inhalation exposures	Monitor ≥3 times a day for ≥3 consecutive, representative days; collect at least duplicate samples for all analyses
To estimate the confidence interval around a mean exposure	Collect ≥6 samples
To estimate the variance of a data set	Collect ≥11 samples

tion, through transport, to receipt at a laboratory (Madsen, 1991). The components of a chain of custody may include (a) attaching a sample label and seal, (b) recording data in a field log book, (c) completing a chain-of-custody record form, (d) filling in a sample analysis request sheet, (e) delivering a sample to a laboratory, (f) receipt and logging of the sample at the laboratory, and (g) assignment of the sample to a laboratory technician for analysis. At the laboratory, the chain of custody continues through the analysis and reporting processes and includes a note of the final storage location of any remaining sample material or the date and manner of its disposal. Reasons for maintaining chain-of-custody records for samples are (a) to ensure that field and laboratory personnel do not lose track of or exchange samples, (b) to prevent tampering with samples, (c) to track mishandling that might compromise a sample's integrity, and (d) to qualify laboratory results as evidence in legal cases.

5.4 Summary

Table 5.11 expands the steps in Table 5.2 for collecting air samples for biological agents and outlines the components of a sampling report. Often, assessments of possible bioaerosol-related hazards aim to describe (a) what biological or other agents may be present, (b) the environmental conditions contributing to their presence, (c) how the agents may affect building occupants, (d) the means by which the bioaerosols reach these human receptors, and (e) what can be done to prevent exposure. Chapter 13 describes how data collected during an investigation can be summarized and analyzed. Chapter 7 discusses how investigators interpret environmental data

TABLE 5.11. Steps in Bioaerosol Sample Collection

Preliminary Data Gathering

- Visit test site.
- Identify the purpose of testing, determine data to be collected, contact laboratory personnel, and select sample collection and analytical methods (see Table 5.7).
- Select sampling locations and time patterns (see Tables 5.4 and 5.5). Determine the number of samples to be collected (see Table 5.9).

Sample Collection

- Schedule sampling activities and assemble equipment. Make sample labels and label sample containers. Prepare sample data sheets.
- Calibrate sampler airflow rates, check for air leaks, prepare blank samples, and conduct other quality assurance tests.
- Run preliminary tests, analyze preliminary data, and modify procedures, as needed.
- Collect samples. Check samples and sample containers. Repeat questionable samples.

Sample Analysis

- Check sample integrity and completeness of sample data sheets. Analyze samples.

Data Reporting

- Assemble results. Perform necessary calculations. Prepare tabular and graphical summaries of sample data.
- Write report. A report may include a transmittal letter, title page, table of contents, list of figures, list of tables, brief summary, and the following sections
  - Introduction
  - Background, purpose, and objectives
  - Materials and methods
  - Test results
  - Discussion of results
  - Conclusions
  - Recommendations
  - References
  - Appendices — copies of all field data, sample and analytical results, field observations, chain-of-custody affirmations, calibrations, and names of field and laboratory personnel.
- Present results to clients relative to the hypotheses that were being tested, and decide a course of action.

TABLE 5.10. Sample Information (adapted from Harding et al., 1996)

- Collector's name
- ✓ Date and time of sample collection
- Study site (e.g., street address or building name)
- ✓ Sample identification number
- ✓ Sample type (e.g., air, surface, liquid, or bulk material)
- Sample collection site: mark location on a map or drawing of the test area, or photograph site with sampling equipment in place
- Number of persons and types of activities at sampling site
- ✓ Sample transportation method and conditions, sample storage conditions
- ✓ Type of analysis requested
- ✓ Date and time samples received at laboratory

Air Samples

- ✓ Sampler type (model number)
- Air sampler and sampling pump identification numbers
- ✓ Sampling air flow rate, sampling start and finish times, volume of air collected

As needed:

- Indoor and outdoor air temperature, RH, CO_2 concentration, and so forth
- Weather conditions, barometric pressure, wind direction and velocity

Material and Water Samples

- Sample temperature, pH, turbidity
- Sample amount or volume
- Total amount, volume, or area of contaminated material at sampling site

✓ Information that should be transmitted to the analytical laboratory along with samples

on specific biological agents to answer the questions posed at the beginning of a study.

5.5 References

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Appendix 5.A. Use of Randomization to Select Sampling Times or Sites

A simple random sample is one in which all possible items have the same probability of being selected for testing. Random selection of sample collection sites and times as well as the sequence in which samples are collected is critical for representative testing. Likewise, laboratory personnel may need random selection methods to examine or analyze samples in an unbiased and representative manner. Occasionally, investigators have little choice in where, when, or in what order they collect samples, but whenever possible, selection should be based on less arbitrary criteria and not solely on judgement or convenience. When in doubt, completely randomized selection may be a good way to minimize bias.

A standard method for random selection of units for testing begins with assigning a number to all units in the population of test units. For example, an 8-hour day could be divided into 20-min segments, numbered from 1 through 24. Likewise, 24 possible sampling sites could be listed in any order and assigned the numbers 1 through 24 (see Column A, Index Number, in Table 5.A.1). Any available method can be used to generate 24 random numbers (Column B). For example, many computer programs and hand calculators can generate random numbers. The random numbers are then sorted from smallest to larges (Column C) while retaining their original index number (Column D). This relisting indicates the order in which the 24 units are chosen for testing. For example, if five time or sites are to be tested, sampling would be conducted a the first five in Column D (i.e., those numbered 5, 17, 24 12, and 4). If all units are to be tested, they would be sample in the order in which they appear in Column D Investigators should not re-use a random number set, bu should generate new sets for every sampling series.

Random sampling may not always be possible o practical. For example, if the sites available for sampling cannot be identified in advance or the duration of a work period is not known, it may not be feasible to list all possible sampling locations or times and assign them random numbers before beginning to collect samples. In such cases a systematic or periodic sampling design may be better (e.g. selection of every fifth office down a hallway from a random starting point or collection of samples for the first 15 min of each hour). Systematic sampling may also ensure tha samples are spread over an entire time period, whereas random sampling can lead to clumps and gaps. Investigators are cautioned that such systematic sampling may be biased if there is a regularity or periodicity to exposure or contaminant presence that the systematic pattern misses.

TABLE 5.A.1. Example: The Randomization Process

Index Number (A)	Random Number (B)	Sorted Random Number (C)	Sample Order (D)
1	7012	0475	5
2	9103	0727	17
3	7622	2378	24
4	2625	2470	12
5	0475	2625	4
6	7361	2727	20
7	3282	3282	7
8	6326	3653	11
9	7564	4364	21
10	9910	4777	23
11	3653	6316	18
12	2470	6326	8
13	9826	6515	16
14	7227	7012	1
15	7534	7227	14
16	6515	7361	6
17	0727	7534	15
18	6316	7564	9
19	8847	7622	3
20	2727	7665	22
21	4364	8847	19
22	7665	9103	2
23	4777	9826	13
24	2378	9910	10

Chapter 6

Sample Analysis

6.1 Introduction

This overview chapter on sample analysis outlines what investigators can realistically learn from air, bulk, and surface samples assayed for various biological agents. Such samples are used qualitatively and quantitatively to (a) identify biological agents and understand the environmental conditions that lead to their presence indoors, (b) demonstrate possible pathways from environmental sources of biological agents to workers, and (c) measure worker exposure to bioaerosols and learn about exposure–response relationships. This chapter briefly describes how microorganisms are classified taxonomically and the primary methods used for their identification. Also discussed are some of the useful features, as well as limitations, of traditional culture methods. Newer analytical techniques are introduced that may cir-

cumvent some of the shortcomings of traditional procedures. These approaches are particularly useful for identifying certain environmental fungi and bacteria and for studying microbial communities. stigators need this kind of information to understand how organisms survive in indoor environments and how their multiplication can be controlled. Table 6.1 in Section 6.8 outlines the assay methods available for various biological agents and the information these methods provide.

6.1.1 Choosing an Analytical Method

Among the first questions investigators ask about indoor environments are: "What types of biological agents are present?" and "How much of each agent is present?" Analytical methods can generally be categorized as one of three approaches, which will be referred to as broad, indicator, and focused methods. Often, microorganisms (e.g., environmental fungi or bacteria) are the biological agents of primary interest but an investigation has not yet targeted any specific microorganisms. For these investigations, *broad analytical methods* that can identify a range of microorganisms are needed. In other cases, particular biological agents are of interest but, for various reasons, an analytical method for an indicator of the agent is chosen rather than a method to detect the agent itself. These are referred to as *indicator methods*. Occasionally, information is needed on one or a few specific biological agents, which narrows an investigation's focus. Therefore, analytical methods for specific biological agents are called *focused methods*.

6.1.1.1 Broad Methods Many investigations of indoor environments aim to evaluate the microbial status of a space. While this goal is never fully achieved, such investigations seek information on the types and concentrations of microorganisms that are present to determine if they appear to be typical or unusual. In these cases, investigators choose methods for sample collection and analysis that provide the broadest possible information. At present, culture-based methods are used most commonly in broad studies and will be described in some detail in this and other chapters. Direct microscopic examination of sample material is also widely applicable for the identification of many fungi. Methods that assess microbial diversity in an environment are increasingly used in research settings. These approaches rely on molecular detection methods and chemical analyses to describe microbial populations and to monitor changes in microbial communities.

6.1.1.2 Indicator Methods Besides microorganism culture and direct microscopic examination of fungi, another means of evaluating the microbial status of an environment is to measure quantitative indicators of the presence of large groups of microorganisms. Examples of this approach are the specific identification of *Escherichia coli* (as an indicator of contamination with raw sewage), the analysis of the glucan or ergosterol content of samples (as a measure of fungal biomass), or the detection of guanine as an indicator of dust-mite presence. Elsewhere in this book, the term "indicator" is used in a similar context when referring to microorganisms or chemical markers whose detection may reflect the simultaneous occurrence or presence of the actual biological agents responsible for adverse health effects (e.g., allergens, toxins, or other cell products) [see 6.7]. Some fungi have also been suggested as indicators of excessive moisture or health hazards [see 19.5.1 and 19.5.3.2]. Indicator methods are relatively straightforward and provide general information on the types of biological agents in an environment. However, these assays may not provide information that is sufficiently specific to make correlations with some types of health effects because these methods measure an indicator or surrogate for a biological agent rather than the agent responsible for a health effect.

6.1.1.3 Focused Methods A third type of investigation seeks to document the presence of specific biological agents associated with particular health effects. For example, when studying an outbreak of Legionnaire's disease, an investigator would use analytical methods specific for detecting legionellae in environmental and clinical samples. Typical analyses would include culture to isolate the bacteria from environmental water samples and specimens from potentially infected persons. Nonculture-based methods are also used to detect the bacteria in environmental and clinical samples (e.g., direct fluorescent antibody stains and polymerase chain reaction). Blood samples could also be drawn to determine if persons with a history of an illness compatible with legionellosis had elevated antibody titers to *Legionella* spp. Similarly, if the specific health effects of aflatoxin were of concern, an investigator would use an assay method suitable for detecting and quantifying that toxin. Methods that focus on single organisms, agents, or health outcomes include nucleic acid probes, chemical assays, and bioassays. The decision to use a focused analysis requires that investigators hypothesize that specific biological agents may be present. Such hypotheses may be based on observed health effects (associated with specific agents) or environmental conditions (that lead investigators to suspect the potential presence of specific biological agents).

6.1.2 Biases in Analytical Methods

6.1.2.1 Biases in Culture-Based Methods All of the broad methods that are currently available for analysis of biological agents are biased in some way. Culture is the most commonly used method for assessing broad groups of microorganisms in indoor environments, but

this method misses fungi and bacteria that are not culturable. Some of the culture methods in popular use to detect and count microorganisms in environmental samples identify such limited subgroups of microorganisms that one might question if these methods may not be more misleading than helpful. Culture-based methods allow detection of only those organisms that are alive, in a condition to grow in culture, and able to successfully compete with the other organisms in an environmental mixture. It is possible (and perhaps likely) that the majority of microorganisms in a particular sample are not identifiable with culture-based methods. The species most readily cultured from a given environment may not be the most prevalent or the most important species present.

The method by which microorganisms were collected may also affect microorganism culturability [see 11.2.3]. Several researchers have studied the effects of air sample collection on the ability of microorganisms to be isolated in culture (Juozaitis et al., 1994; Stewart et al., 1995; Terzieva et al., 1996). Collection methods that expose bacterial cells to drying (e.g., filters or dry cyclones) are generally not suitable for analyses that require that bacteria be viable (Jensen, et al., 1992). However, these sample collection methods may be appropriate for analytical approaches other than culture [see Table 11.1].

6.1.2.2 Biases in Microscopic Examination of Environmental Samples Microscopic examination of spore-trap samples can provide an accurate estimate of total fungal spore concentrations, a broad picture of the kinds of spores in an air sample and, when combined with culture-based analysis, quite accurate estimations of fungal populations. However, used alone, microscopic examination of air samples allows specific identification of only a few fungal species, and most spores must be placed in general categories [see 19.4.2]. Direct microscopic examination of source samples may allow identification of individual fungi, but this method is not usually quantitative and does not allow identification of unstained bacteria. Alone, neither culture nor microscopy provides an accurate assessment of the spectrum of biological agents in environmental samples. Together, these methods can provide relatively complete information on fungal populations. For use with bacteria, investigators must decide which genera and species are of interest and select appropriate stains and viewing techniques [see 6.4 and 18.4].

6.1.2.3 Biases in Analytical Methods Other than Culture and Microscopy In various ways, analytical methods other than culture or microscopy also restrict the information that can be obtained about biological agents in environmental samples. Chemical, immunological, and biological assays can be useful indicators or focused tools. To be used as broad methods, these assays require the development of extensive data libraries for agent identification. Development of such databases requires that the potenti constituents of the biological populations of interest b known, which is not always the case.

6.2 Microorganism Classification and Identification

When biological agents are examined in environmenta samples, it is often important to identify the organism (more or less specifically) to allow comparisons of the agent present in different locations or at different times. All liv ing organisms (bacteria, fungi, plants, and animals) ar identified in the context of some previously agreed upo classification scheme. Such systems define the characteris tics of organisms that are grouped together at different lev els of similarity, beginning with kingdoms and proceed ing to species. There is no universally accepted definitio of a "species," which is simply the taxonomic group tha subdivides a genus (Stahl, 1997) [see Chapter 17].

In large part, classification depends on morphologi cal and physiological similarities and differences amon related organisms, such as those described in th sections on *Characteristics* in the chapters on bacteria, fung amebae, viruses, and house dust mites. Members belong ing to the same classification or taxon share certain ke characteristics (i.e., combinations of features or proper ties that are unique to a group). Key characteristics ma be morphological, physiological, biochemical, immuno logical, or molecular. Whole dust mites, amebae, and som fungal spores and fungi in culture can be readily identi fied by microscopic examination. However, identifying bacteria to genera and species generally requires subcul turing and biochemical testing of the pure cultures. En vironmental isolates that do not exactly match the ke characteristics listed in reference texts may present iden tification problems. Therefore, making a reliable identifi cation of the entire range of microorganisms that may be found in environmental samples can be a time-consum ing undertaking. Before sampling, investigators shoulc carefully consider what level of organism identificatior they will need to test a study's hypotheses and develop recommendations for control and remediation.

6.2.1 Prokaryotic Microorganisms

Bacteria and archaea are prokaryotes (Gr. before cells) and are usually classified based on evolutionary (phylogenetic) relationships. Observed similarities in characteristics of morphology (physical size and shape) and physiology (processes and functions) are also used for categorization [see Chapter 18]. Many classification schemes for bacteria have been proposed over the years, and it is important to note that there is no "official," universally agreed upon system. One of the best known and most widely accepted classification systems is that described in *Bergey's Manual of Systematic Bacteriology* (1989). In this scheme, classification is based, in part, on the probability of matching defined morphological, physiological, and

TABLE 6.1. Collection and Analysis of Environmental Samples for Biological Agents (for acronyms and abbreviations see list)

Biological Agent	Primary Sample Collection Methods	Sample Analysis	Data Obtained	Chapter
AMEBAE	Bulk (water) Air: impingers, impactors	Culture Microscopy: cell morphology Bioassay: immunoassay — labeled antibody stains	Concentration: number per m³ or mL Isolate identification Confirmation of the presence of a specific ameba	20
ALLERGENS/ANTIGENS (cockroach, mammalian, avian; see also bacteria, fungi, and dust mites)	Bulk (settled dust)	Bioassay: immunoassay (ELISA)	Concentration (allergen/antigen): µg/g or units of allergen/g or antigen/g	25
BACTERIA	Air: impactors, impingers, wetted cyclones, filters Bulk/Surface samples	Direct microscopy/total counts Bioassay: immunoassay — labeled antibody stain Molecular assay: nucleic acid probe (molecular hybridization), nucleic acid amplification (PCR) Culture: colony morphology Microscopy following culture: cell morphology, Gram-stain characteristics Biochemical assay following culture: substrate utilization assay	Concentration: cells per m³, g, or cm² Confirmation of the presence of a specific bacterium Confirmation of the presence of a specific bacterium Concentration: CFU per m³, g, or cm² Isolate identification (general) Isolate identification (specific)	18
Bacterial antigens	Bulk (settled dust)	Bioassay: immunoassay (ELISA)	Concentration (bacterial antigen): µg/g	25
Bacterial cell-wall components: Endotoxin (Gram-negative bacteria) Other (total bacteria)	Air: filters, impingers Bulk (settled dust) Air: filters Bulk (settled dust)	Bioassay: LAL Chemical assay: GC-MS or HPLC Chemical assay: GC-MS or HPLC	Biological Activity (endotoxin): endotoxin units per m³ or g Concentration (LPS): ng per m³ or g Concentration (muramic acid, diaminopimelic acid - PG): ng per m³ or g	23
Bacterial whole-cell lipids; phospholipids	Air: filters, impingers Bulk: surface samples	Chemical assay: GC, GC-MS	Community profile	18
FUNGI	Air: impactors, filters, impingers, wetted cyclones	Direct microscopy/total counts	Concentration: spores per m³, g, or cm²; spore identification	19
Bulk/Surface samples Microscopy following culture: spore and hyphal morphology	Culture: colony morphology	Concentration: CFU per m², g, or cm² Isolate identification		
Yeasts (whole-cell lipids, fatty acids)	Air: impactors, impingers, wetted cyclones Bulk/Surface samples	Chemical assay: GC Biochemical assay: substrate utilization assay	Isolate identification	19

TABLE 6.1. Collection and Analysis of Environmental Samples for Biological Agents (for acronyms and abbreviations see list) (cont.)

Biological Agent	Primary Sample Collection Methods	Sample Analysis	Data Obtained	Chapter
Fungal allergens	Bulk (settled dust)	Bioassay: immunoassay (ELISA)	Concentration (fungal allergen): ng or units per g	25
Fungal cell-wall components	Air: filters Bulk samples	Bioassay: LAL, immunoassay Chemical assay: CG-MS, HPLC	Biological activity (glucan): units or µg per m^3 Concentration (glucan or ergosterol): units or µg per g	24
Fungal toxins	Air: impactors, filters, impingers, wetted cyclones Bulk samples	For toxigenic fungi: (see Direct microscopy, Culture, and Microscopy following culture, above) For toxins:		19
		Chemical assay (TLC, HPLC, GC-MS); Bioassay: immunoassay, cytotoxicity assay	Confirmation of toxin presence; Concentration (toxin): ng per m^3 or g Confirmation of toxin presence Detects toxic activity without toxin identification	24
DUST MITES	Bulk (settled dust)	Microscopy: mite morphology	Concentration (number mites): per g of dust or m^2 of sampled surface Mite identification	22
Dust-mite allergen	Bulk (settled dust)	Bioassay: immunoassay (ELISA)	Concentration (mite allergen): ng per g of dust or m^2 of sampled surface	
Guanine (marker)	Bulk (settled dust)	Chemical assay: LC, colorimetric	Concentration (guanine): per g of dust or m^2 of sampled surface	
VIRUSES	Air: impingers, cyclones, impactors, filters	Bioassay: cell culture, immunoassay — labeled antibody stains Electron microscopy Molecular assay: nucleic acid probes (molecular hybridization), nucleic acid amplification (PCR)	Concentration (cytopathic units): number/m^3; Isolate identification Confirmation of the presence of a specific virus Isolate identification Confirmation of the presence of a specific virus	21
MVOCs	Air: sorbent or whole air sampling	Chemical assay: GC-MS	Concentration (compound): mg/m^3 Compound identification	26

phenotypic characteristics, the latter being visible properties of an organism that are produced by interactions between a genotype and the environment [see 18.1.2]. Considerable laboratory effort may be needed to identify a bacterium isolated from the environment. Occasionally, it is not possible to assign a given organism to any described genus; other times, an isolate may be assigned to a genus, but it may not be possible to make a definite species identification.

6.2.2 Eukaryotic Microorganisms

The eukaryotic organisms (Gr. true cells) of interest in investigations of indoor environments include many fungi, a few amebae, and the plants and animals that produce allergens responsible for building-related hypersensitivity diseases [see Chapters 19, 20, 22, and 25]. Fungi may reproduce both sexually and asexually but are generally classified by the morphology of their sexual structures [see 19.1.2 and 19.1.3]. The sexual and asexual forms of a fungus may differ morphologically and even have different names. These multiple names are a potential source of confusion in laboratory reports and the literature. Fungal isolates that do not sporulate in laboratory culture may be very difficult or impossible to identify. In addition, fungal characteristics used for identification may vary depending on growth conditions. In the past, for routine bioaerosol investigations, fungi often were identified only to the genus level. Increasingly, investigators are recognizing the importance of identifying fungi to the species level [see 19.4.1]. Such identification is becoming more widely available from commercial laboratories, but some identifications require the experience and expertise of a mycologist who has specialized in the study of a particular fungal group.

6.2.3 Viruses

Viruses are classified according to the structure and arrangement of their proteins and by the kind of nucleic acid (DNA or RNA—but never both) in their cores [see 21.1]. Viruses are not included in routine bioaerosol characterizations, in part, because the detection and identification of viruses require very specific methods and may be difficult. However, modern molecular methods have reduced the necessity for viral cultivation. Specifically designed molecular probes now permit the screening of environmental samples for some viral agents, primarily in water and soil samples but increasingly in air samples [see 6.6].

6.3 Fungal and Bacterial Culture

Culture-based assays for fungi and bacteria involve providing a growth environment that encourages the microorganisms in a sample to multiply. For air samples, this is often done by collecting the bioaerosols directly onto agar-based media in culture plates or by inoculating plates with small portions of air samples collected in a liquid. For source samples, culturing is done by directly inoculating culture plates with measured portions of dust or liquid samples or by making suspensions of bulk or surface samples and plating portions of these.

Knowing how to optimize the information that culture-based methods can provide requires familiarity with the range of fungi and bacteria that may be found in indoor environments. The *Sample Analysis* sections of the chapters on bacteria, fungi, amebae, and viruses describe how microbiologists identify microorganisms that have been grown in laboratory culture. Analysis of culture data is discussed in Chapter 13.

6.3.1 Preparation of Environmental Samples for Culture Analysis

Air samples are possibly the most common type of environmental sample that investigators collect to study bioaerosols. Investigators should know what type of sample analysis they plan to use when collecting source samples so that the collection procedure is compatible with the requirements of the analytical procedure. Typical source samples are settled dust, pieces of potentially contaminated material, water samples, and surface washes or contact samples. Preparation of source samples for assays other than culture may be similar to the methods of sample preparation described here for culture-based analysis.

6.3.1.1 Agar-Impaction Samples No processing is needed prior to incubation for air samples collected by direct impaction onto agar-based culture media. However, laboratory personnel should examine plates upon receipt and note any growth that occurred prior to incubation. Laboratory personnel should also look for debris on the agar surface or evidence of excess moisture or agar dehydration that could invalidate samples.

6.3.1.2 Liquid Samples Liquid samples (e.g., bulk water samples, impinger and cyclone fluids, and surface and bulk material washings) may be plated directly on the culture media of choice. If bulk samples are viscous or semisolid (i.e., sludge like) or if liquids were collected from heavily contaminated areas, it may be necessary to make serial dilutions in sterile buffered saline or diluted broth prior to plating. Such dilutions are usually made in multiples of ten (i.e., 10^{-1}, 10^{-2}, and so forth).

6.3.1.3 Bulk Material and Surface Samples Dust samples may be sieved and weighed portions (e.g., 30 mg) inoculated directly onto the surfaces of culture media where the samples are spread with a sterile glass rod or other implement. Dust samples and bulk samples of solid items

(e.g., contaminated fabrics, carpet, wallboard, ceiling tile, or insulation material) may also be weighed and washed or extracted in sterile buffered saline or diluted broth. Addition of a wetting agent, such as 0.01% Tween 80 (J.T. Baker Inc., Phillipsburg, NJ), helps to separate microbial cells from each other and from material to which the cells may adhere. Washings or suspensions are processed as liquid samples and may be diluted prior to plating. More types of culturable fungi have been isolated when dust samples were plated directly onto agar than when the same dust was suspended in a liquid before plating. However, overgrowth and interference is more common with the direct dust inoculation method than with suspension and dilution methods. Suspending bulk samples in liquid and making serial dilutions of the suspensions has been found to yield higher estimates of the concentration of fungi in bulk samples, probably by reducing problems with overgrowth and enhancing spore or cell separation.

6.3.2 Culture Media

There are many varieties of culture media and many specialized uses for them. Some media are fairly general, that is, able to support a large variety of microorganisms, sometimes both fungi and bacteria. Other media are selective, differential, or both. A *selective medium* is formulated to give some microorganisms a physiological or nutritional advantage over others. Some nonselective media can be made selective by adding antibiotics to inhibit the growth of certain microorganisms. A *differential medium* may support the growth of more than one microorganism but contains ingredients that produce differences in the appearance of various fungi or bacteria. An example of a medium that is both selective and differential is the eosin-methylene blue agar used to isolate and differentiate enteric bacteria (i.e., those found in the intestines). Gram-positive bacteria (GPB, e.g., *Staphylococcus aureus*) will not grow on this medium. Among the Gram-negative bacteria (GNB) that will grow, *Escherichia coli* produces blue-purple colonies with a metallic sheen whereas *Shigella sonnei* colonies are colorless. Selective and differential media form the basis of the classic methods used to identify bacteria [see 18.4.1.3].

In a general survey of environmental microorganisms, the culture medium of choice would likely be a nonselective, broad-spectrum medium able to support the growth of a wide variety of microorganisms. Soybean-casein digest agar (also known as tryptic or trypticase soy agar), nutrient agar, and R2A are examples of nonselective media used to culture bacteria. Various formulations of malt extract agar are broad-spectrum media often used for fungi. Rose bengal agar is a selective medium that favors the multiplication of some slow-growing fungi from environments such as soil, in which many rapid-growing fungi are also present. This medium contains a dye that inhibits bacteria and some rapid-growing fungi. Fungi adapted to dry conditions (xerotolerant or xerophilic fungi) may r quire a medium such as DG18 (with dichloran and gly erol) or other medium with high solute concentratic that limits the amount of available water. Tables 18 and 19.5 provide the formulations for these media. Ol viously, an investigator's choice of a culture medium a critical part of a sampling and analysis plan. There no agreement on the best all-purpose medium for isc lating either total culturable fungi or bacteria. Invest gators and microbiology consultants can refer to the li erature on environmental sampling and check other re erences for direction on choosing culture media for spe cific purposes (Atlas, 1993; AIHA, 1996a; ASM, 1997).

6.3.3 Incubation Conditions

6.3.3.1 Incubation Temperature Microorganism growing in indoor environments are generally adapte for growth at ambient temperatures. Most fungi grov optimally over a temperature range from 18° to 25°C whether in the environment or in laboratory culture. Th majority of fungi grow poorly, if at all, at temperature above 35°C. A few fungi (e.g., *Aspergillus fumigatus*) grov well at higher temperatures and can be selectively iso lated by incubation at 40°C.

Environmental bacteria also grow well in the 18° t 25°C temperature range, but many bacteria can tolerate tem peratures above 30°C. Cultures for environmental bacte ria are generally held at ~28°C [see 18.4.1.2]. This tempera ture is lower than that typically used in clinical laborato ries for isolating pathogenic microorganisms, which grow at human body temperature. Incubation of environmental samples at ~37°C is only appropriate to isolate bacteria that cause infectious diseases. Cultures for thermophilic actinomycetes are incubated above 50°C, which encourages the growth of these bacteria and excludes nonthermotolerant fungi and bacteria. During sample transport, the multiplication of microorganisms that can grow at room temperature must be prevented by keeping inoculated plates cool enough to inhibit microbial multiplication. Samples should be delivered to the laboratory within 24 hours of collection. Temperature extremes during sample transport and storage can also cause dehydration of water-based media and condensation in culture plates, both of which should be avoided.

6.3.3.2 Incubation Atmosphere Atmosphere is another incubation condition that an analytical laboratory must consider. Most analysis of air samples for culturable microorganisms has been limited to obligately or facultatively aerobic species (i.e., those that require molecular oxygen or those that can grow with or without it). Therefore, no special care is taken to control the atmosphere in which samples are incubated. Essentially all fungi and many bacteria are aerobic. However, anaerobic bacterial species may persist in the environment as spores or as vegetative forms grow-

ing in sequestered areas without oxygen. Selective analysis for anaerobic bacteria requires special sample transport and incubation to exclude oxygen (e.g., anaerobic liquid media in sealed containers or anaerobic chambers or jars for culture plates). Growth of some bacteria is enhanced by incubation in 2.5% to 10% CO_2 (e.g., *Legionella* spp.), and special incubators may be required to maintain these conditions.

6.4 Microscopy

Microbiologists and other biologists use microscopes extensively as described in Sections 18.4.2, 19.4.2, 20.4.3, 22.4.2, and 24.4.1.2. Total counts of fungal and bacterial cells can be made by microscopic examination of environmental air samples. Fungal cells and spores can be counted with or without staining. Many fungi have morphologically distinctive spores and can be identified by direct microscopic examination to the genus level (e.g., *Cladosporium* and *Alternaria* spp.) or even the species level (e.g., *Stachybotrys chartarum* and *Epicoccum nigrum*).

For bacteria in culture, staining is one of the first steps performed to identify cell morphology and staining characteristics. For identification of bacteria by direct microscopic examination (without culture), the information obtained is the concentration of total cells or total cell counts. Fluorescing stains [e.g., acridine orange (AO), 4',6-diamidino-2-phenyl-indole (DAPI), or fluorescein isothiocyanate (FITC)] are often used with epifluorescence microscopy (Atlas and Bartha, 1993; APHA, 1995; Chapin, 1995). Until recently, a disadvantage of some direct bacterial stains was the lack of differentiation among cells of similar size and shape. For example, many rod-shaped bacteria, live or dead, Gram-negative or Gram-positive, appear alike with some stains. However, fluorescent Gram-stain kits are now commercially available as are reagents that differentially stain live and dead organisms (Hensel and Petzoldt, 1995; Madelin and Madelin, 1995; Morris, 1995; Terzieva et al., 1996; Heidelberg et al., 1997; Lawrence et al., 1997). Image processing may facilitate counting of microorganisms in some types of samples (Kildesø and Nielsen, 1997). Labelled antibody stains are useful to identify selected bacteria (e.g., a direct fluorescent antibody stain may be used to detect *Legionella pneumophila* in filtered water samples) (AIHA, 1996b).

6.5 Bioassays

Bioassays are quantitative methods in which the end point is some observable effect on a biological system or organism. In this book, the term bioassay is used in a broad sense to include *in-vivo* and *in-vitro* tests based on biological systems or organisms. Animal and cell culture assays for viruses and bacteria are bioassays as are assays that expose cells or whole organisms to samples that may contain toxins. Immunoassays (which depend on the response of biological molecules, that is, antibodies) and the *Limulus* amoebocyte lysate assay for endotoxin (which depends on the response of a lysate of cells from the blood of the horseshoe crab) can also be considered bioassays.

6.5.1 Infectivity Assays

Viruses cannot function outside of living cells, therefore, laboratory assay for viruses involves inoculating tissue cultures or whole animals with sample material, the former being more widely used [see 21.4]. In a tissue culture, each viral particle results in a focus of infection in the confluent cell layer as evidenced by formation of a plaque or lesion. Tissue culture assays provide information on the number of viral particles that were able to attack the type of cell provided. Applying tissue culture analysis to environmental samples is especially difficult because of the likelihood that other microorganisms present in the samples will also grow and obscure the results. Thus viral culture is limited as a tool for assay of environmental samples.

6.5.2 Immunoassays

Immunoassays are biological assays based on the specificity of the antigen-antibody reaction. This specific affinity has been exploited for a great number of applications, among them the detection of biological agents in environmental samples (e.g., dust-mite, animal, and cockroach allergens; microbial antigens; fungal glucans; aflatoxin; and latex) [see 22.4.3, 24.7.3, and 25.4].

An enzyme-linked immunosorbent assay (ELISA) is a widely-used method to quantify allergen concentration in dust samples. Briefly, the wells of a polystyrene microtiter plate are coated with capture antibodies, which are specific for particular antigens (Ab_1; Step 1, Figure 6.1). Dust samples are sieved to obtain the fine dust fraction, soluble proteins are extracted from the dust in an appropriate buffer, and the extracts are serially diluted. The same is done for an allergen standard, and aliquots of diluted standards and dust sample extracts are added to the antibody-coated wells (Step 2). The plates are incubated and washed to remove unbound materials. Bound allergen is detected with a second enzyme-conjugated antibody (Ab_2) that recognizes a cross-reacting site (Step 3). An enzyme substrate/chromogen solution is added, and the optical absorbance (color change) is read with a microtiter plate reader (Step 4). The concentrations of target antigens are obtained by comparing the optical density readings for dust samples with standard curves of optical densities generated from allergen standards of known concentration. Assays for more than one allergen (e.g., fungus, cockroach, dust mite, bird, cat, dog, or rodent allergens) can be performed on the same dust suspensions by substituting appropriate antibodies (IOM, 1993) [see 25.4].

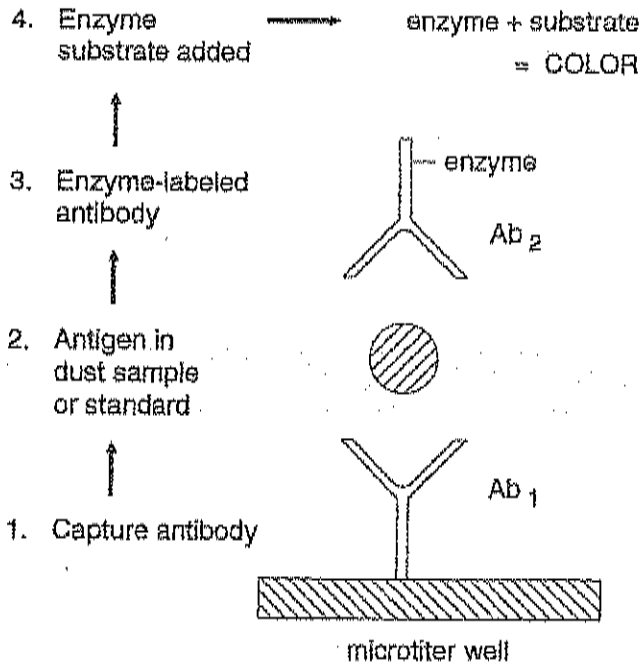

FIGURE 6.1. Enzyme-linked immunosorbent assay (ELISA).

6.5.3 Bioassays for Toxicity

The *Limulus* amoebocyte lysate assay is a bioassay in which the observable effect is a clot, increase in turbidity, or a chromogenic reaction. This method is used primarily to measure endotoxin concentrations but has also been used for fungal glucans [23.4.2 and 24.7.3]. Cytotoxicity assays are used to detect materials toxic to cells [see 24.4.3]. For example, the brine shrimp assay is used to assess the relative toxicity of purified toxins or environmental samples that may contain toxins. The endpoint measured in this test is the death of these microscopic animals. Various other tests are available to identify compounds that are mutagenic in bacterial or mammalian cell assays. These assays can be used on specific compounds or mixtures of compounds.

6.6 Polymerase Chain Reaction (PCR)

6.6.1 The PCR Process

Sometimes, rather than a survey of all microbial species in an environment, what is needed is a method to screen samples for a specific genus or species [see 6.1.1.3]. Polymerase chain reaction (PCR) is an example of such a method. PCR can be very sensitive and specific and has seen application for detecting the presence of some airborne infectious agents. Advantages of PCR over culture are its speed (results can be obtained in a matter of hours rather than days or weeks) and its ability to detect difficult-to-grow, slow-growing, and even nonculturable microorganisms (Gao and Moore, 1996; Buttner et al., 1997).

PCR is based on *in-vitro* replication of selected nucleic acid sequences and, theoretically, can copy and recopy the DNA from a single organism millions of times. DNA consists of two complementary strands with specific nucleotide bases always pairing from opposite pieces of a genome (A in Figure 6.2). The strands separate (B) and are copied, with each strand acting as a template to replicate its complement (C).

The PCR process uses temperature to separate two DNA strands (B in Figure 6.2). Enzymes (polymerases) are added along with the appropriate nucleotide bases to be used as building blocks for the copying. If the appropriate physical and chemical conditions are repeated in a fixed cycle, the strands are repeatedly copied, separated, and recopied, resulting in an exponential increase in the number of identical DNA pieces. In a few hours, there is enough material for analysis. Nucleic acid probes are short nucleotide sequences able to bind (hybridize) with species-specific regions of targeted DNA (Figure 6.3). Specialized probes are tagged with radioactive or fluorescent labels, and these labelled probes are permitted to react with the amplified DNA. A probe will bind in detectable quantities if the organism of interest was present in the initial sample and a targeted segment of its DNA was successfully amplified.

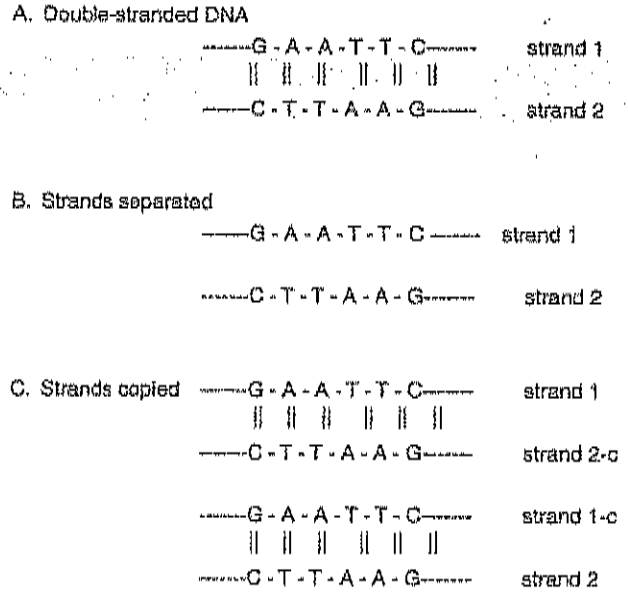

FIGURE 6.2. Short section of a DNA sequence showing paired nucleotide bases and DNA replication.

6.6.2 Use of PCR for Environmental Sampling

PCR has been used for clinical diagnosis of certain infectious diseases without the necessity of culturing the responsible pathogens. Some clinical PCR tests have been adapted to also detect infectious agents in environmental samples. Likewise, PCR systems designed for environmental water samples have been used to detect specific

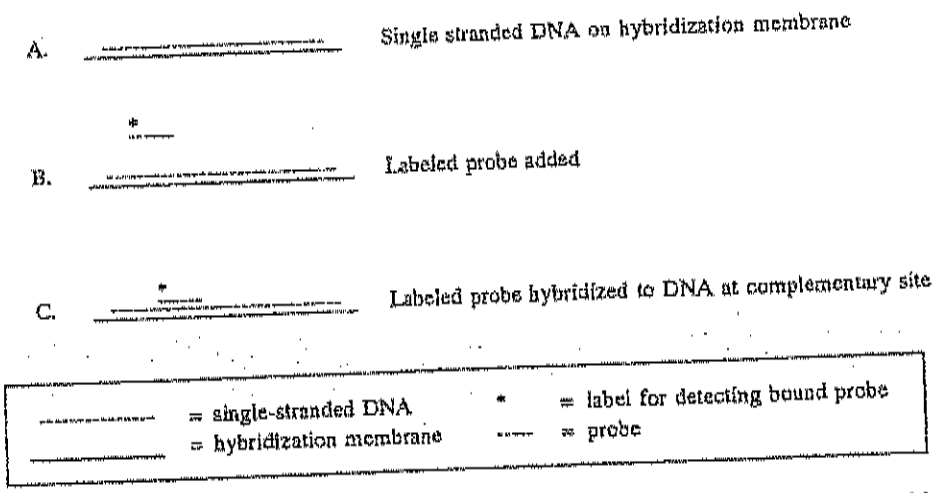

FIGURE 6.3. Illustration of hybridization of a DNA probe to a complementary section of a nucleic acid sequence.

organisms in air samples collected with impingers, cyclones, filters, or other apparatus (Alvarez et al., 1994; Mukoda et al., 1994; Palmer et al., 1995; AIHA, 1996b; Mastorides et al., 1997).

The PCR method is especially useful for the rapid detection of organisms that are difficult or impossible to culture in the laboratory. It is also an alternative method for the detection of organisms for which culture and manipulation might pose special hazards for laboratory personnel. The PCR method amplifies DNA from live or dead organisms. A variation of the method permits the analysis of gene expression, which provides information about the biological activity of microorganisms as well as their presence.

At the time of this publication, some of the limitations of the method are that it is a focused method and only predetermined agents can be detected, if present. PCR is occasionally subject to interference from compounds found in some environmental samples. The method is not as widely available as other analyses, being more a research than investigative tool, and is primarily qualitative (a measurement of presence or absence) rather than quantitative (a measurement of the exact amount of the target organism). Despite these limitations, PCR and other forms of nucleic acid amplification are finding increasing use in environmental microbiology, and new methods appear in the literature as investigators adapt procedures for wider applications (Amann et al., 1995; Podzorski and Pershing, 1995; Stahl, 1997). PCR can be used to analyze bulk material, water, soil, and bioaerosol samples for the presence of specific microorganisms or for environmental surveillance of indicator organisms.

6.7 Chemical Assays

Dissatisfaction with analytical methods that depend on the growth of microorganisms in laboratory culture has led to the application of the tools and methods of analytical chemistry to organism identification. High performance liquid chromatography (HPLC), gas chromatography (GC), and mass spectrometry (MS) are used—singly or in combination—to identify certain "chemical markers." Chemical markers are biological molecules that may be either components or products unique to certain groups of microorganisms (Fox et al., 1990). These markers include (a) structural molecules (e.g., carbohydrates such as peptidoglycans from bacterial cell walls and beta-glucans from fungi), fatty acids (components of bacterial cell walls), lipids (ergosterol from fungal membranes), and lipopolysaccharides (endotoxin from GNB), which contain both lipid and carbohydrate moities, (b) metabolic products (e.g., mycotoxins from fungi, alcohols, aldehydes, and other volatile organic compounds, from bacteria and fungi) (c) secreted macromolecules (e.g., specific enzymes and other proteins), and (d) excreted macromolecules (e.g., guanine in dust-mite fecal pellets). Depending on the specific method used, the information derived may be general (e.g., measurement of glucan or ergosterol to estimate total fungal biomass), agent specific (e.g., chemical assays for specific mycotoxins), or microorganism specific (e.g., determination of fatty acid profiles to identify specific bacteria). Methods for analysis of some of these compounds and the kinds of data these assays can provide are discussed in Sections 19.4.3, 22.4.4, 23.4.3, 24.7.3, and 26.5.

Much effort is currently being invested in the development of analytical microbiology. However, at this time, the work is being performed primarily in research laboratories, and the commercial availability of such testing is limited. The development of computer-directed automation of some of the methods may result in simpler, reproducible protocols for instrumental analyses of chemical markers. Fatty acid analysis allows measurement of total biomass in a sample (a quantitative description of community structure) and does not require that organisms be culturable or even viable (Macnaughton et al., 1997; White et al., 1997). GC has been used to analyze the methyl ester derivatives of fatty acids from whole-cell extracts. This method

was evaluated for identification of bacteria from the family *Micrococcaceae*, which often comprise a major fraction of culturable airborne bacteria in buildings (Pendergrass and Jensen, 1997). Some investigators have used carbon-source analysis to characterize microbial communities based on metabolic profiles and made inferences about community diversity (Garland and Mills, 1991).

6.8 Sampling and Analytical Methods

As described above, a wide assortment of analytical methods are available to investigators to study biological agents in indoor environments. Occasionally, multiple methods are available for a single biological agent (e.g., a bioassay and a chemical assay). Table 6.1 summarizes the information in the *Sample Analysis* sections of the chapters on specific biological agents in Part III of this book. The table lists biological agents, methods for sample collection, methods for sample analysis with the corresponding information investigators can gain from the analyses, and the chapters that discuss these methods. Note that more than one sample collection method may be listed for each biological agent. The listed collection methods apply to all of the analytical methods that follow for a particular agent. For example for amebae, both bulk water samples and air samples can be analyzed by culture, microscopy, and bioassay. Note, however, that the column on data obtained for each analysis is listed for a particular analysis. Respectively, the three analyses listed for amebae in water or air samples provide information on organism con-centration (culture), isolate identification (microscopy), or confirmation of the presence of a specific ameba (bioassay).

6.9 Qualifications of Laboratories and Laboratory Personnel

Few IHs, EHPs, or IEQ consultants have laboratories capable of analyzing bioaerosol samples. Therefore, commercial laboratories are employed to analyze most environmental samples for biological agents. Investigators should contact a laboratory before collecting samples to ensure that the laboratory can handle the samples in a timely manner when received. Until investigators gain experience and confidence in bioaerosol sampling, laboratory personnel should participate in the study design phase of investigations and in the development of sample collection plans. Such participation will help ensure that investigations are conducted reasonably and in accordance with current recommendations from appropriate professional associations.

6.9.1 Choosing an Analytical Laboratory

Many factors contribute to an investigator's choice of what type of analytical laboratory to use and selection of a particular laboratory within a chosen type. Among the factors that may influence this choice are (a) the services the laboratory can provide, (b) what the laboratory charges for analyses, (c) proximity of the laboratory to the sampling location, (d) availability of rapid sample transport, and (e) turn-around time for delivery of laboratory reports. However, equally important are the laboratory's qualifications to conduct the requested analyses, their record of past performance, and the willingness of the laboratory personnel to work with an investigator in designing a sampling plan and interpreting test results.

At a minimum, a laboratory must have the necessary equipment and staff to carry out the tests requested. For example, if asked to identify indoor and outdoor bacteria, a laboratory should have the basic equipment needed to incubate and identify bacteria and should provide the services of a qualified environmental bacteriologist. Likewise, if fungal samples are to be analyzed, it is necessary that an experienced environmental mycologist be available to examine them. Laboratories should have written analytical methods and procedures, and laboratory personnel should keep careful and complete records of what tests they perform and the results. In addition, quality assurance programs should be routine in all laboratories, and written policies and records of compliance should be available [see 5.3 and 13.4].

6.9.2 Laboratory Proficiency

As requests for indoor air analyses have increased, more laboratories have offered bioaerosol testing. Until recently, most laboratories conducting environmental microbiology analyses performed only the most basic tests (e.g., the fecal coliform test to assess water quality). There are certification programs to evaluate such laboratories, but the criteria on which water testing is judged may not be appropriate for evaluating bioaerosol analysis. The American Industrial Hygiene Association (AIHA, Fairfax, VA; Laboratory Accreditation Department: 703-849-8888) accredits laboratories for testing metals, asbestos, silica, and organic solvents to help investigators locate laboratories that have demonstrated their ability to analyze workplace samples accurately. In 1996, AIHA began the Environmental Microbiology Proficiency Analytical Testing (EMPAT) program with participants from the U.S. and Canada. The program is designed for laboratories specializing in detection of microorganisms commonly found in air, fluids, and bulk samples. Plans are that the EMPAT program will lead to an accreditation program for environmental microbiology laboratories; however, at the time of this publication, no laboratories have been accredited.

Until laboratory certification procedures are in place for laboratories analyzing environmental samples for biological agents, testing laboratories should participate in appropriate programs for performance evaluation or proficiency testing. If no such programs are available, laboratories should consider conducting inter-laboratory evaluations in collaboration with other researchers and commercial testing groups.

6.9.3 Quality Control in the Laboratory

Quality control during sample analysis in the laboratory is as important as quality control during sample collection in the field. Laboratory personnel should clearly understand an investigators record-keeping requirements and should be prepared to assume responsibility for samples placed in their custody [see 5.3]. The ability with which investigators can draw accurate conclusions from observations and measurements depends on the quality and defensibility of their data. Data defensibility involves the use of proper procedures (written protocols that include descriptions of the laboratory's quality control practices); protection of samples from inappropriate alteration; use of proper record collection, handling, and security; and accurate documentation of all sample-related information. Investigators often submit field blanks and duplicate samples to check the quality of sample handling in the field and sample analysis in the laboratory [see 5.3, 6.9, 7.2.2, and 13.4].

A laboratory is responsible for samples after they are received from the field, but not before that point. A laboratory may include a disclaimer in a test report to limit the laboratory's liability for inaccurate results or inappropriate use of the data they supply because of their lack of control over the method of sample collection, the age or condition of samples when received, or the limitations of the analytical methods used. The disclaimer may state that the field investigator is ultimately responsible for interpretation of test results because the laboratory personnel did not see the sampling site first hand and were not present when the samples were collected [see 2.1.4].

6.10 Summary

Many analytical methods are available to investigators to study biological agents in indoor environments. However, there clearly is still much to be accomplished to improve the assessment of indoor biological contaminants, demonstrate the pathways by which bioaerosols travel from sources to workers, and measure worker exposures. Many newer assay methods are useful for analyzing and characterizing microorganisms in bulk samples. However, some of these methods do not have the sensitivity required to analyze air samples. Some methods may offer sensitivity, but lack specificity, whereas others may be both sensitive and specific, but not quantitative. At the time of this publication, it is still very much the task of a field investigator or expert consultant to assess each unique situation and determine, first, if sampling is needed and, if so, which sample collection and analysis methods would provide the best answers to the questions the circumstances pose. Therefore, it is important that field investigators clearly communicate their specific needs and goals to the laboratory personnel with whom they work. Precise objectives will help ensure design of the best possible study, development of suitable protocols for sample collection and analysis, optimal use of resources, and accurate interpretation of the findings.

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Chapter 7

Data Interpretation

7.1 Introduction

Data interpretation should be considered when developing an investigation strategy and designing a sampling plan. In turn, investigation strategies and sampling plans evolve after investigators have compiled enough information to formulate hypotheses to explain available facts and have determined that environmental sampling or an epidemiological study are required. Ideally, investigators establish the criteria by which they will judge samples for biological agents before collecting them. Criteria for interpreting environmental sampling results can be drawn from reference documents and research publications. Identifying the criteria by which data will be judged can help investigators determine the usefulness of the data they propose collecting. Anticipating potential test outcomes can help investigators determine if they have sufficiently clear and defensible criteria for judging test results and if the data they propose collecting will provide a firm basis for further decisions that may need to be made.

7.2 Rationale and Assumptions

7.2.1 Data Collection

"Data" may consist of the simple observation of fungal growth on a wall, analytical measurements from hundreds of environmental samples, or the results of a survey of building occupants with and without particular building-related conditions. During building evaluations, investigators may collect observational data, data on building performance, and bioaerosol data. Investigators gather observational data when they see visible sources of biological agents or observe environmental conditions that may allow the growth of microorganisms or arthropods. To collect information about a building's performance, investigators often measure such environmental parameters as temperature, humidity, air movement, and pressure. Investigators may collect air or source samples to gather bioaerosol data.

7.2.2 Data Quality

The ability of qualified persons to draw accurate conclusions from observations and measurements depends in large part on the quality and reliability of the original data. Some of the criteria used to judge data quality are (a) representativeness, (b) completeness, (c) reproducibility, (d) accuracy, (e) precision, and (f) integrity. Data are not useful if their quality cannot be assessed because investigators cannot be sure what weight to give the information. For example, the interpretation of a single air sample is difficult without information on the variability of the concentrations of biological agents in the sampled environment. One of the greatest difficulties with design and interpretation of bioaerosol studies is the fact that variability in the measurement of airborne contaminants is almost always large. Most approaches to bioaerosol sampling involve collecting short-term, grab samples which, alone, provide no information on the variability of the concentration of the agent being measured. Some bioaerosol samplers allow time-discriminated or time-weighted-average sampling. However, such sampling is uncommon in indoor environments because of the longer sampling times involved (e.g., hours to days rather than minutes). Among the most difficult decisions investigators face is identifying what environmental data they need and then determining if they have the time and resources to obtain data of sufficient quality to meet their needs. If adequate means are not available, investigators must decide if they will collect data of lesser quality and trust that it will be interpretable in some meaningful way. Alternatively, investigators may decide not to collect environmental samples using instead other information (e.g., observational data) to support their conclusions and recommendations.

7.2.3 Data Analysis

Data analysis is the step where an investigator (a) checks and validates data (i.e., reviews it for consistency and to identify particularly striking observations), (b) reduces or summarizes data (i.e., compiles the results in tables or figures and calculates summary statistics), and (c) estimates the data's reliability and looks for statistically significant associations and differences. The ultimate goal in data analysis is to test the validity of the hypotheses the investigators have formulated about the study site. For example, in an HP outbreak, an investigation team may have hypothesized that disease is related to exposure to thermophilic actinomycetes. The investigation team gathers environmental data in areas with and without HP cases and analyzes the data to decide if there is a demonstrable and significant difference between the case and control environments. This information may help the investigators decide how to improve the work environment and reduce workers' risk for HP.

Investigators should carefully consider the criteria by which they will judge environmental sampling data and information from epidemiological surveys. It may be difficult to demonstrate statistically significant differences in relative concentrations of biological agents or prevalence rates for specific symptoms. Therefore, investigators should not allow failure to demonstrate statistical significance to persuade them to ignore suspected hazards. Conversely, statistical significance does not automatically imply relevance or importance in the real world in terms of worker health and safety. There may be real differences between the types and relative concentrations of biological agents in environments or the rates of symptom reporting in groups of workers. However, these differences may not indicate that one environment is less safe than the other or that one group of workers is being affected by a biological agent or other hazard in their environment. The challenge for an investigator is to apply knowledge, expert advice, training, and experience when analyzing data and to recognize the strengths and limitations of various types of data and methods of data analysis.

7.2.4 Data Interpretation

Data interpretation is the step where investigators make decisions on (a) the relevance to human exposure of environmental observations and measurements, (b) the strength of associations between exposure and health status, and (c) the probability of current or future risks. These interpretation steps are followed by decisions on what measures can be taken to interrupt exposure and prevent future problems. Table 7.1 summarizes these steps for three examples. Case A illustrates the interpretation of data that is primarily observational. Case B presents air sampling data collected for an evaluation of a single worker with HP, who may be a sentinel case indicating a need to evaluate co-workers. Case C discusses the interpretation of environmental sampling data when epidemiological and medical data are also available.

For the examples in Table 7.1, several questions, possible interpretations, and alternative outcomes are presented. The possible interpretations illustrate that a study may reach more than one conclusion and investigators should be prepared to handle whatever interpretation the data best support. Among the possible outcomes are "yes, this agent from this source is responsible for adverse health effects," or "no, there is no evidence that this environment is a source for disease-causing agents." Note that the statement "these data indicate the tested environment is safe" cannot be made. Data may support the assertion that an environment is "safe" (i.e., presents little or no risk of exposure to biological agents), but investigators cannot claim to have proven this.

7.3 Approaches to Data Interpretation

Figure 7.1 illustrates the factors necessary for an exposure to a biological agent to result in a response, for example, a symptom or a disease. Beneath each primary

TABLE 7.1. Example: The Interpretation Process for Several Kinds of Data

Data	Questions Addressed	Possible Interpretations	Possible Outcomes
A. Surface contamination (possibly fungal growth) observed in a building in which the occupants complain of allergic symptoms	What is the chance the observation is not true (i.e., that the material is not microbial growth or is not releasing bioaerosols)? How representative is the observation of the general environment?	The material is microbial contamination and is releasing bioaerosols. The growth is likely to be producing biological agents that are known to cause BRI. The growth is relevant to human exposure and existing disease.	Yes, the evidence appears to support these relationships. No, the evidence does not support these relationships. The evidence is insufficient to reach a conclusion.
B. Results of 100 air samples for culturable thermophilic actinomycetes in a workplace in which a case of HP has been confirmed	What is the nature of the variability within the data set? What are the qualitative characteristics of the data set (i.e., what kinds of bacteria are present; are any thermophilic actinomycetes present at unusual or unexpected concentrations)?	Thermophilic actinomycetes are present in sufficient quantity to cause disease. There are statistically significant patterns that could explain disease distributions. The data represent an unusual and undesirable exposure. The data satisfactorily explain the existence of disease.	Yes, the data represent an unusual exposure. Yes, the data explain the existence of disease. Yes, the data represent an unusual exposure, but do not explain disease. No, the data do not support these relationships. The data are insufficient to reach a conclusion.
C. Five documented cases of Legionnaires' disease; *Legionella pneumophila* recovered from a water source that may produce aerosols	What are the chances that five cases of Legionnaires' disease would occur by chance among the occupants of a building of this type? Is there commonality among the cases with respect to (i) *L. pneumophila* type or (ii) possible exposure? Do any of the *L. pneumophila* isolates from patients match those from environmental sources?	Water in an environmental source is supporting the *L. pneumophila* serogroup that was isolated from one or more patients. There is a logical pathway linking a suspected source and infected workers. The data support bacterial transmission from a specific environmental source. The epidemiological and environmental evidence is sufficient to link the outbreak to the building.	Yes, the infections are building related and the probable source has been identified. Yes, the infections are building related, but the source has not been identified. Yes, a source has been identified, but is not clearly associated with disease. No, the data do not support a building-related source for the apparent outbreak. The data are insufficient to reach a conclusion.

factor are related parameters that determine if the progression can continue and if the outcome will be a change in health status.

Ideally, documentation that an agent causes a particular disease includes identification of (a) an agent, (b) its immediate, local source and environmental reservoir, and (c) exposure sufficient to cause the response observed. Data may consist of the identification of a source (observational data), measurement of the concentration of a biological agent at the source (bulk sampling data), or measurement of the concentration of the agent in air at a particular time (air sampling data). To establish a clear connection between an exposure and an outcome, an investigator must work through all the steps in Figure 7.1. Unfortunately, data are seldom available on release of biological agents from sources, bioaerosol decay, airway deposition of bioaerosols, agent release to the body from inhaled and deposited particles, or the human factors determining host response. Further, so little is known about these factors for most biological agents that extrapolation from available general information to the specifics of a given case may be uncertain. However, good quality data from environmental investigations can be interpreted as being representative of environmental conditions, and extrapolation of these data to indicate exposure is also often possible, as discussed in the next sections.

7.3.1 Dealing with Visible Microbial Growth

Various authors have recommended that microbial growth in occupied interiors, in HVAC systems, and on building materials and furnishings, especially if extensive, should be avoided and that any contamination that exists should be removed and further contamination should be prevented (Samson et al., 1994; Health Canada, 1995; Marcmi et al., 1995; ISIAQ, 1996). "Extensive" visible fungal growth has been defined as surface areas greater than 3 m^2 (32 ft^2) (NYCDH, 1993; Health Canada, 1995; ISIAQ, 1996).

It is well established that bioaerosols cause infectious and hypersensitivity diseases and that bioaerosols in the indoor environment may cause toxic effects, although data on inhalation exposure is limited. Therefore, it is reasonable to use indicators of environmental contamination as a basis for evaluating the need for improved maintenance or remediation in a preventive context. It is also reasonable to use this approach as a means of making decisions in response to outbreaks of BRIs and BRSs. However, caution should be taken in ascribing specific causal links, as described in Chapter 14. At the time of this publication, there is no scientific basis for applying specific exposure limits for concentrations of total or specific culturable or countable bioaerosols (see 1.2).

7.3.2 Comparison with Existing Standards or Guidelines

In the U.S., no federal agency has clear authority to regulate exposure to biological agents associated with BRIs. The OSHA General Duty Clause and Hazard Communication Standard have been used to resolve IEQ problems, for example, to protect remediation workers and building occupants during clean-up operations and to inform building occupants of probable exposure to significant amounts of potentially harmful biological agents (Morey, 1992). The situation may differ in other countries and investigators should be familiar with federal and local regulations relating to bioaerosol exposures.

Workplace exposure limits are based on epidemiological and measurement data (Vincent, 1995). Epidemiological studies examine dose–response relationships and lead to health-based exposure criteria. The dose needed to produce a given response must be related to many factors, for example, the air concentration of an agent, the time period workers are exposed to the agent, the deposition and retention of particles within the respiratory tract, the concentration of the active agent at the target tissue, and the potency of the agent. Measurement-based studies used to establish exposure criteria provide comparative or relative data for problem and control environments. From comparisons of the resulting data, exposures are derived that may be considered acceptable, tolerable, or unlikely to cause harm. If available, data from studies of controlled human or experimental animal exposures may also be considered in the establishment of limit values.

By far, comparison of an environmental measurement with an existing standard is the simplest method to interpret data. Providing data are collected in the manner that

ENVIRONMENT		HOST	
SOURCE → OF AGENT	EXPOSURE → TO AGENT	DOSE → OF AGENT	RESPONSE TO AGENT
Dissemination of agent	Air concentration of agent Time spent in environment	Breathing rate Particle deposition Release of biologically active agent	Host sensitivity/ susceptibility Adequacy of host defenses

FIGURE 7.1. Steps connecting a biological agent and a host response.

was used to establish a standard, a measurement and a standard can be compared directly and an immediate determination can be made as to whether the sample exceeded the limit. However, there are no standards that specify acceptable concentrations for airborne materials of biological origin other than those that have been studied in certain manufacturing environments (see 1.2). Guidelines and recommendations are available for bioaerosol control in the commercial biotechnology industry and microbiology laboratories (USDHHS, 1993; WHO, 1993; Sayre et al., 1994), but these recommendations have little bearing on other indoor environments.

Although numerical guidelines and recommendations have been published (e.g., for total concentrations of fungi and bacteria), these criteria vary over orders of magnitude. No consensus, health-based guidelines exist nor are any likely to be developed until more data are available on dose-response relationships for specific agents and health outcomes and more baseline data have been collected from randomly selected environments. The reader is referred to Rao et al. (1996) for a review of guidelines on bioaerosol exposure, to Health Canada (1995) for a review of guidelines for mycological indoor air quality, and to Maroni et al. (1995) for a discussion of IEQ guidelines and standards. Other references provide recommendations for controlling moisture problems (ISIAQ, 1996), assessing and remediating indoor fungal growth (NYCDH, 1993), and interpreting environmental sampling data (AIHA, 1996). Some baseline data on typical, acceptable, or tolerable bioaerosol concentrations may result from the analysis of data such as that from the USEPA Building Assessment Survey and Evaluation (BASE) studies of non-problem office buildings (USEPA, 1994).

Until guidelines on acceptable concentrations of biological agents are developed for particular environments and human populations, it is imperative that investigators not invoke previously published numbers, most of which the original authors no longer support. Although, ACGIH has given numerical guidelines for data interpretation in earlier documents (ACGIH, 1986; 1987; 1989), at the time of this publication, ACGIH does not support any existing numerical criteria for interpreting data on biological agents from source or air samples in non-manufacturing work environments. Instead, ACGIH recommends gathering the best data possible and using knowledge, experience, expert opinion, logic, and common sense to interpret information and design control and remediation strategies.

> Although, ACGIH has previously published numerical guidelines, at the time of this publication, ACGIH does not support any existing numerical criteria for interpreting data on biological agents from source or air samples in non-manufacturing environments.

7.4 Interpretation of Data on Biological Agents

The following sections briefly describe interpretation of data on air or source samples for the primary biological agents to which workers in non-manufacturing occupations may be exposed. These sections are brief summaries of the data interpretation sections of the individual chapters in Part III, which provide more complete discussions for specific agents and references for the information included here.

7.4.1 Bacteria

Few guidelines or recommendations are available to interpret data on bacterial aerosols. The information obtained from environmental sampling for bacteria may help investigators determine if environmental bacteria are multiplying in a building and, if so, may identify their source. Sampling may also help investigators determine if human-source bacteria are present at concentrations that should cause concern and if the kinds and relative concentrations of bacteria identified indicate a risk to health.

7.4.1.1 Bacteria from Outdoor Air Some bacteria that are common in outdoor air may penetrate to building interiors and may also grow indoors. Decisions on whether identification of bacteria and bacterial agents in indoor air represents simple entry with outdoor air or actual growth in the indoor environment depend on the relative indoor and outdoor concentrations. The dominance of a single species may reflect indoor growth, whereas the presence of mixed bacterial species may reflect intrusion of outdoor air or shedding from building occupants. Actinomycetes (especially thermophilic ones) are relatively rare in indoor and outdoor air. Therefore, the presence of actinomycetes in indoor air may be considered an indication of an indoor source.

7.4.1.2 Human-Source Bacteria Human-source bacteria typically dominate indoor air in occupied buildings and are abundant in dust and on surfaces. Air concentrations of these bacteria depend on the number of persons present, their activity levels, the types of clothing they wear, and the ventilation rate to the space. Extremely high concentrations of human-source bacteria in low-activity spaces may indicate overcrowding or inadequate ventilation.

7.4.1.3 Gram-Negative Bacteria (GNB) A predominance of GNB in an indoor environment may suggest the presence of sources that would be considered unusual in clean, properly ventilated buildings. For example, bacteria typical of fecal contamination may be found where plumbing has leaked or toilet exhausts are improperly vented. However, finding a few of these bacteria mixed with normal human-source organisms is not a cause for concern. Contamination of standing water with GNB is common and may be a concern if the bacteria produce odors or can become aerosolized. Dominance of GNB in air may suggest the presence and aerosolization of contaminated water.

7.4.1.4 Significant or Substantial Health Risk Some information is available on concentrations of infectious bacteria that have been associated with disease outbreaks. For example, elevated concentrations of *Legionella* spp. in cooling towers have been linked epidemiologically with disease outbreaks. Likewise, tuberculosis transmission has been related to source strength and ventilation rate. However, little is known about the potential effects for healthy adults and children of exposure to the majority of bacteria recovered during routine air and source sampling. Often, it is only possible to establish that an unusual exposure situation does or does not exist with respect to a control environment.

7.4.2 Fungi

Many fungi produce allergens and some fungi produce toxins. Fungal growth in buildings is undesirable and may cause health problems for building occupants. Although it may be difficult to establish that exposure to fungal aerosols occurs or that exposure presents a hazard, indoor fungal growth is inappropriate and should be removed. Further, steps should be taken to correct conditions that led to fungal growth so that it does not recur. Visible contamination that is confirmed by source sampling to be fungal growth is evidence of indoor contamination. Air sampling (culture or spore-trap sampling) may also indicate indoor fungal growth but should be followed by inspection and source sampling to identify the location of fungal contamination.

In the presence of the inevitable background concentration, the challenge for environmental sampling is to detect indoor fungal growth or entry of fungal aerosols from sources near OAIs and to document the contribution of such sources to occupant exposure. Interpretation of possible indoor fungal exposure has been addressed using (a) indoor/outdoor total concentration ratios, (b) comparisons of the species compositions indoors and out, and (c) the presence of indicator species in the indoor environment.

7.4.2.1 Indoor/Outdoor Comparisons The concentration of fungi in indoor air typically is similar to or lower than the concentration seen outdoors. Exceptions are enclosed agricultural and other specialized environments (where indoor fungal concentrations may be much higher). Outdoor concentrations may exceed those measured indoors even where indoor fungal growth is obvious. If outdoor fungal concentrations are very high, indoor/outdoor concentration ratios for total fungi may be low, even in the presence of significant indoor growth. On the other hand, outdoor fungal concentrations may be reduced during times of snow cover or other conditions that suppress the release of fungal spores from outdoor sources, at which times, indoor measurements may be higher than those outdoors even in the absence of significant indoor sources. Finally, if the variability of the data is high (which is common), extensive sampling may be required to establish that two locations differ. The species of fungi found in indoor and outdoor air typically are similar if outdoor air is the primary source for the fungi in indoor air. Comparisons of the species compositions of indoor and outdoor populations requires accurate identification of fungal species not simply identification to the genus level.

7.4.2.2 Indicator Species Fungi whose presence may indicate excessive moisture or a specific health hazard have been termed indicator organisms. Interpreting the presence or absence of an indicator species requires the ability to identify fungi to the species level and a knowledge of the prevalence of the indicator species in both indoor and outdoor environments. The mere presence of a few CFUs or spores of any fungus should be interpreted with caution. Identification of a particular fungus in an indoor environment does not allow an investigator to conclude that building occupants are being exposed to allergenic or toxic agents. Investigators should also recognize that fungi that have been named indicator species are not the only fungi of significance. Many fungi other than those specifically listed by various groups may cause problems for building occupants exposed through inhalation of aerosols or by other contact.

7.4.2.3 Potentially Pathogenic (Infectious) Fungi Some fungal pathogens should be assumed to be present when materials known to support their growth are found (e.g., *Histoplasma capsulatum* and *Cryptococcus neoformans* in bird and bat droppings). Removal of such materials should be conducted as if they contained pathogenic fungi. Disturbance of soil or other material that may contain fungal pathogens (e.g., compost containing *Aspergillus fumigatus* or material enriched with bird or bat droppings) should be conducted with consideration that occupants of neighboring buildings may be exposed if airborne fungal spores enter the buildings.

7.4.3 Amebae

Investigators should be aware that amebae can cause inhalation fever (e.g., humidifier fever), severe eye and wound infections, and fatal encephalitis, although these conditions are rare. The size of amebic trophozoites and cysts causes them to fall rapidly from air and greatly diminishes the risk of infection when these amebic forms are discharged as aerosols. Physicians should consider the presence of pathogenic amebae when a patient's infection does not respond to traditional antibiotic treatment. Potential environmental sources of amebae shou be tested for pathogenic types if infections are identifiec These tests should be conducted by laboratories with experience in the assay of pathogenic amebae. Matching

of amebae from clinical specimens and environmental samples may implicate a water supply as the source of an infection.

7.4.4 Viruses

The number of virus particles required to cause infection in a susceptible individual is not known for most viruses. However, evidence suggests that for some viruses one particle may be sufficient to initiate infection. The data retrieved from environmental sampling is usually the number of viruses in a sample that are able to infect cultured cells or laboratory animals. This number is not necessarily the same as that needed to infect a human host. Therefore, data from such samples may indicate only (a) the presence and concentration of the virus, or (b) the probability of the absence of the virus. Investigators must establish their own criteria for interpreting such data with respect to risks for humans.

7.4.5 Environmental Allergens

A lack of well-characterized, standardized reagents poses problems in the testing of environmental samples for the presence of many antigens (which stimulate the production of antibodies) or allergens (which are antigens associated with hypersensitivity disease). The precision of immunoassays for indoor allergens is rather poor and sample variation can be large. In addition, people have varying sensitivities to allergens. Therefore, investigators should not interpret too strictly comparisons of recommended allergen thresholds and actual environmental measurements.

7.4.5.1 House Dust Mite Allergens Limits for dust-mite allergen concentrations in settled dust have been proposed for residences. Exposure to dust containing 2 µg/g of group-1 mite allergen (roughly equivalent to 100 mites/g or 0.6 mg/g of guanine) is considered to increase the risk of dust-mite sensitization as well as the development of asthma and bronchial hyperreactivity in affected persons. Exposure to dust containing 10 µg/g of group-1 mite allergen or >2.5 mg/g of guanine represents an increased chance of acute asthma attacks. Epidemiological studies have found better correlations between symptoms and ELISA measurements of mite allergen than mite counts. However, measurement of dust allergen content on a per-gram basis may be misleading if diverse substrates are compared because the density of dust from various sources may differ markedly (cf. dust from bedding or upholstered furnishing, which contains lint and fine feathers, and carpet dust, which contains soil, sand, and coarse fibers).

7.4.5.2 Fungal Allergens Over 60 species of fungi are known to produce allergens that cause allergic rhinitis (hay fever) and asthma, and many more are probably also involved. Purified and characterized allergens have been prepared for *Alternaria alternata*, *Aspergillus fumigatus*, and *Cladosporium herbarum* and a few other fungi. However, none of these allergens has been adequately tested for the analysis of environmental samples. To date, the limited analysis of fungal allergens in indoor environments precludes estimation of a risk level for symptom exacerbation or even determination of what is a high level. Researchers have also attempted to determine threshold concentrations of fungal spores associated with immunological sensitization or symptom development. While there may be threshold concentrations of fungal allergens below which no symptoms are experienced, this is probably not an absolute value but a gradient based on individual sensitivities and the kind of fungus.

7.4.5.3 Bacterial Antigens Exposure to bacterial allergens has been associated with work-related asthma, HP, humidifier fever, and a disease resembling ABPM. Like fungi, bacteria secrete enzymes that can act as allergens. *Bacillus* spp. are used to produce proteases that are added to laundry detergents for stain removal. ACGIH has adopted a ceiling limit for subtilisins (100% pure crystalline enzyme) of 0.00006 mg/m^3 (ACGIH, 1997). However, data on bacterial allergens is limited, many bacterial allergens are poorly characterized, and exposure to bacterial allergens in non-manufacturing indoor environments is interpreted on a case-by-case basis.

7.4.5.4 Cockroach Allergens Cockroaches are the only insects repeatedly recognized as a common source of indoor allergens. However, significant levels or thresholds of cockroach allergen exposure have not been determined. Any cockroach allergen detected in an environment (i.e., any amount above the LDL of the assay used) identifies an indoor area that places cockroach-allergic persons at some risk for symptoms and places allergy-prone (susceptible) persons at some risk for sensitization and symptom development.

7.4.5.5 Cat and Dog Allergens Cat and dog allergens have been detected in schools and offices where these animals were not kept, suggesting that persons with animal contact may carry allergens on their clothing. Moderate to high concentrations of cat allergen (≥1 µg *Fel d* 1/g dust) have been found, most often in the offices of persons who have cats at home. *Fel d* 1 concentrations above 8 µg/g have been proposed as a threshold for sensitization. Fewer persons appear to be sensitive to dog than to cat allergens, perhaps because more dogs are housed outdoors and owners may bathe dogs fairly frequently whereas cats generally groom themselves. Concentrations of dog allergen (*Can f* 1) sufficient to result in the development of symptoms have not been established. In one study, *Can f* 1 concentrations ranged from <0.3 to

23 µg/g in dust from houses without dogs and from 10 to 10^4 µg/g in those with dogs.

7.4.6 Endotoxin

Environmental endotoxin measurements from different laboratories are not necessarily comparable because the *Limulus* amebocyte lysate (LAL) assay is a comparative rather than analytical method and because of the lot-to-lot variation in LAL reagent sensitivity. At present, it appears that only data from one laboratory assaying samples from one environment while using one LAL lot can be considered entirely comparable. Experimental studies of human exposure to cotton dust and the majority of recent field studies suggest an endotoxin threshold for acute airflow obstruction in the range of 45 to 330 EU (endotoxin unit)/m^3. Thus, most current data support a threshold for acute airflow obstruction within a 10-fold range. These values are generally 10 to 100 times the background endotoxin levels reported in the studies.

For the time being, the imprecision of the LAL assay over time and among laboratories makes it impossible to establish a TLV at a given endotoxin level. Therefore, ACGIH proposes a practical alternative approach for limiting endotoxin exposure using relative limit values. This approach is based on comparison of endotoxin activity levels in the environment in question with simultaneously determined background levels. Investigators must ensure that background endotoxin levels are determined under appropriate and representative conditions.

Relative limit values (RLVs) are proposed because of the observation that endotoxin levels between 10- and 100-fold higher than background are frequently associated with adverse health effects. Therefore, it should be assumed that endotoxin plays a role and action should be taken to reduce exposure when (a) there are health effects consistent with endotoxin exposure (e.g., fatigue, malaise, cough, chest-tightness, and acute airflow obstruction), and (b) endotoxin exposures exceed 10 times simultaneously determined, appropriate background levels. Thus, 10 times background is proposed as an RLV action level in the presence of respiratory symptoms. In environments with a potential for endotoxin exposure but no current complaints, endotoxin levels should not exceed 30 times the appropriate background. Thus, 30 times background is a maximum RLV in the absence of symptoms. When exposures exceed the RLV action level or maximum RLV, appropriate remedial actions should be taken. Other than as described here, it is premature to make recommendations regarding low-level endotoxin exposures such as may occur in homes and offices.

7.4.7 Peptidoglycan (PG)

Sampling and analysis for PG is an interesting area worthy of further research. However, there currently is no significant body of data relating airborne PG exposure to health effects. Therefore, guidelines for interpreting sampling results and recommendations regarding the significance of observed PG concentrations cannot be made.

7.4.8 Fungal Toxins

Investigators may encounter six indicators of potential exposure to mycotoxins:

1. Observation of fungal growth in an indoor environment.
2. In source samples, identification of fungi known to produce mycotoxins.
3. In air samples, identification of fungi known to produce mycotoxins.
4. Identification of mycotoxins in source samples.
5. Identification of mycotoxins in air samples.
6. Detection of biomarkers for mycotoxins or fungi in biological specimens (e.g., urine, breast milk, or blood).

The first of these provides the weakest evidence of possible mycotoxin exposure; the next five provide progressively stronger evidence, with the possible exception of biomarkers. Specific associations between any of these indicators and any illness cannot be made. However, all may indicate inappropriate conditions that should be corrected. See Section 7.4.2 for a discussion of the interpretation of data in the first three of these categories.

7.4.9 β-(1→3)-D-Glucans

Exposure to some concentration of β-(1→3)-*D*-glucans can be assumed whenever fungi are isolated from an environment. β-(1→3)-*D*-glucans have been proposed as agents possibly responsible for some cases of BRSs. However, it is difficult to imagine how BRSs could routinely be associated with such ubiquitous agents unless other factors also play a role. Even in problem environments, fungal concentrations (and presumably, although not studied, β-(1→3)-*D*-glucan concentrations) are likely lower than concentrations frequently encountered outdoors. Workers exposed to high concentrations of β-(1→3)-*D*-glucan (during drybagging of the compound produced for use as a food additive in processed cheese) showed no irritant symptoms. In studies that have noted associations between BRSs and glucans, it is possible that glucan measurements were acting as surrogates for exposure to other fungal products. It is also possible that exposure to a mixture of fungal products (including glucans) such as may occur in some indoor environments plays an important role in the development of BRSs.

7.4.10 Microbial Volatile Organic Compounds (MVOCs)

Studies indicate that many microorganisms produce VOCs some of which may also be released from nonmicrobial sources. A few MVOCs (e.g., 1-octen-3-ol, 3-methyl-1-butanol, 2-hexanone, and 2-heptanone) may originate specifically from fungi but not solely from any

single fungus. Other MVOCs (e.g., ethanol, which microorganisms may produce in large amounts) have many additional non-microbial sources. The fraction of the total VOC burden in buildings that may arise from microorganisms is not known, but it can be expected to vary with the nature and extent of indoor microbial growth and the presence of other sources. The few available laboratory measurements indicate that microorganisms may contribute significant amounts of VOCs. However, at the time of this publication, no simple, rapid, inexpensive, and reliable method is available to identify the nature and extent of indoor microbial growth from measurements of indoor VOC concentrations. Also, no library is available that gives MVOC profiles for combinations of microbial species and substrates. Some degree of fungal and bacterial growth can be found in most indoor environments and humans also contribute VOCs. Researchers need to distinguish typical MVOC emissions from such background sources.

7.5 Summary

Chapter 2 describes how investigators gather information with which to formulate hypotheses, collect data to test these hypotheses, and draw conclusions from the information they have compiled. This chapter summarized key points in the interpretation of data on biological agents. The separate chapters in Part III elaborate on these points for the individual agents. Chapter 13 describes how data are handled to facilitate interpretation and Chapter 14 describes how investigators use experimental, epidemiological, and environmental data to establish cause–effect relationships for exposures and responses.

Of the types of data outlined in Figure 2.4 (environmental, bioaerosol, medical, and epidemiological data), this chapter primarily described interpretation of bioaerosol data. Investigators who need to interpret environmental data should review Chapters 4 and 10. Investigators may need to consult an engineer, architect, or HVAC specialist for advice and direction on conducting and interpreting tests of building performance. Investigators who need to interpret medical or epidemiological data should review Chapters 3 and 8. Investigators may also need to consult a physician, toxicologist, or epidemiologist for advice and direction on evaluating medical or epidemiological data.

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Chapter 8

Medical Roles And Recommendations

Cecile S. Rose with Kathleen Kreiss, Donald K. Milton, and Edward A. Nardell

8.1 Background

Medical personnel can play important roles in identifying bioaerosol-related illnesses and responding to them. Medical expertise should be sought for (a) diagnosis and management of an individual worker with a possible building-related illness (BRI), typically building-related hypersensitivity disease, inhalation fever, or infection, and (b) investigations of populations of workers with building-related symptons (BRSs). Medical personnel usually participate as members of a team that may include an IH, EHP, IEQ consultant, engineer, epidemiologist, or toxicologist. In the first circumstance, diagnostic confirmation of a BRI is a sentinel health event that raises concern for others with shared building exposures. Such events that involve serious morbidity, mortality, or risk to the community may require notification of public health authorities. In the second circumstance, investigation of symptomatic building occupants may require medical surveillance and/or diagnostic evaluation.

This chapter outlines the medical evaluation of possible bioaerosol-related conditions and provides guidelines for physicians and other health-care professionals on (a) identifying and managing BRIs and BRSs due to bioaerosol exposure, and (b) the appropriate roles of health-care professionals in investigating such complaints. The health effects of exposure to specific biological agents are also discussed in the individual chapters of Part III.

8.2 Approach to Patients with Building-Related Illness (BRI)

8.2.1 Hypersensitivity Diseases

Hypersensitivity (allergic) respiratory diseases are among the most common illnesses associated with bioaerosol exposures in the indoor environment. Untreated, hypersensitivity diseases can cause significant decrements in workers' productivity and quality of life and can even be life threatening. A complete medical history—including information on possible workplace, residential, and recreational exposures (Table 8.1)—is the first step in accurate diagnosis and management of hypersensitivity diseases (Menzies and Bourbeau, 1997). In addition, physicians use a number of diagnostic tools to assess the presence of hypersensitivity diseases caused by bioaerosols [see also 25.2.3].

A key factor in hypersensitivity diseases is that disease is not apparent with the first exposure to an antigen (allergen). A latency period is required, during which the immune system becomes sensitized to the antigen [see 25.2.1]. The sensitization process requires three factors (a) a genetic capability to respond to the antigen, (b) sufficient exposure to the antigen or to a combination of the antigen and an immunostimulating substance, and (c) time for the specific lymphocytes and production of effector molecules, such as antibodies [see 25.2.2].

TABLE 8.1. Components of an Occupational and Environmental History for Patients with BRI Possibly Due to Bioaerosol Exposures

Occupational History

- Chronology of current and previous occupations
- Description of job processes and specific work practices
- List of specific chemical, dust, and other aerosol exposures (e.g., grain dust; animal handling; food and plant processing; cooling towers, fountains, and other water sprays; and metal-working fluids)
- Review of Material Safety Data Sheets (MSDSs) to identify known chemical sensitizers and irritants
- Records or reports of industrial hygiene evaluations or environmental testing at the workplace
- Symptom improvement away from work or symptom increase with specific workplace exposures
- Presence of persistent respiratory or constitutional symptoms in exposed co-workers

Environmental and Residential History

- Pets and other domestic animals (especially birds and cats)
- Hobbies and recreational activities (especially those involving chemicals, feathers or fur, plant materials, and organic dusts)
- Presence of humidifiers, dehumidifiers, swamp coolers, clothes dryers vented indoors, and other humidity sources
- Use of hot tubs or saunas
- Leaking or flooding in a basement or attic
- Water damage to carpets or furnishings
- Visible fungal growth
- Feather pillows, comforters, or clothing

8.2.1.1 Rhinitis and Sinusitis In allergic rhinitis, inflammation of the nasal lining causes rhinorrhea (runny nose), congestion, itching, and sneezing. Allergic sinusitis—as a complication of allergic rhinitis—is associated with thickening of the mucosal lining, leading to purulent (puslike) nasal and pharyngeal drainage and cough. Headache, facial pain, earache, sore throat, halitosis, and fever are also common. Rhinitis and sinusitis may be evaluated using rhinomanometry, radiography or CT of the nasal sinuses, and assessment of inflammatory cells in the nasal mucosa. Testing a person before and after exposure to an implicated environment may be useful. Sections 3.3.1 and 25.2.2.1, on allergic asthma, rhinitis, and sinusitis, also address these conditions.

Besides biological agents, chemical irritants can also cause inflammation of the nasal mucosa—leading to rhinitis—as well as irritation of the eyes and upper and lower respiratory tract. Chemical irritants may be inhaled as gases or mists or via particles to which they have adsorbed. Such irritation is generally reversible after short-term exposure. However, temporary tissue damage may make a worker susceptible to irritants that the person otherwise would tolerate. To identify and control exposures, it is necessary to consider all possible causes of nasal and sinus irritation, that is, biological (antigenic), chemical, and physical agents (Kipen et al., 1994).

8.2.1.2 Asthma Diagnosis of asthma is usually made on the basis of symptoms (wheezing, chest tightness, cough, and shortness of breath) and the presence of airway hyperreactivity [see also 3.3.2 and 25.2.2.1]. The latter response is measured by either tests of bronchial hyperresponsiveness or a positive reaction to a bronchodilator on pulmonary function testing. Exposure challenge in the suspect environment, with careful physiological assessment, is useful in determining whether asthma symptoms can be causally linked to a work-related exposure. Workers themselves can collect peak-flow measurements using small, portable instruments. Serial measurements may be useful to document airflow changes. A worker can take peak flow measurements throughout the day, both in and out of the implicated environment (i.e., at work during the day and at home in the evening and on weekends). Serial tests of lung function (such as spirometry measurements made by a trained technician) at the beginning and end of the work week can also be helpful [see 25.2.3.2]. Four patterns of lung function response to occupational exposures are recognized (i.e., equivalent daily decline, weekly decline, first day only decline, and persistent obstruction) (Burge, 1993). A pulmonologist or occupational medicine physician experienced in diagnosis of occupational asthma is often required to make such a diagnosis. In some cases it may be necessary to remove an individual from a work environment before improvement in lung function occurs.

Less informative are laboratory challenge and immunological tests. Laboratory challenge with a suspect antigen is poorly standardized, difficult to interpret, and usually unnecessary to confirm the diagnosis of building-related asthma. Skin prick testing for specific IgE antibodies to common indoor allergens confirms the allergic status of an individual but is seldom helpful in diagnosing building-related asthma. Section 25.2.3.1 describes immunological tests.

As mentioned for rhinitis in Section 8.2.1.1, asthma may also have chemical and physical causes in addition to biological (antigenic) ones (Bernstein, 1997). Therefore, to identify and control asthma-related exposures, it is necessary to consider all possible work-related agents. Skin testing may be useful for diagnosis of allergen-induced asthma but not for identification of irritant-induced asthma.

8.2.1.3 Hypersensitivity Pneumonitis (HP) Clinicians often base the diagnosis of HP on chest radiographs or CT scans, tests of lung function, the finding of specific serum precipitating antibodies, and a history of exposure to materials known to be associated with HP. However, these tests may be normal in early disease, and antibody testing may be negative when the responsible antigen is not among the standard panel of tested substances [see also 3.3.3 and 25.2.2.3]. Referral to a pulmonary disease subspecialist for exercise testing or fiberoptic bronchoscopy with bronchoalveolar lavage and transbronchial biopsy is usually necessary to confirm the diagnosis of HP and should be obtained when symptoms or exposures are compelling (Rose, 1996). Testing a person before and after exposure to an implicated environment may also be useful in the acute form of HP.

8.2.1.4 Medical Management of Hypersensitivity Diseases The pharmacological treatment of individuals with hypersensitivity BRIs does not differ from the management of patients with such illnesses due to causes unrelated to building exposures. Antihistamines, decongestants, intranasal corticosteroids, and nasal saline irrigation are the mainstays of treatment for allergic rhinitis and sinusitis. Chronic asthma is treated with inhaled steroids, bronchodilators, other inhaled medications with antiinflammatory effects, and oral corticosteroids for more severe or acute attacks. HP may resolve completely following removal from exposure without pharmacological treatment, although a course of oral corticosteroids is often used in circumstances of severe or persistent symptoms.

Environmental control strategies to eliminate antigen exposures are key to the medical management of building-related hypersensitivity diseases as these conditions may worsen with continued exposure despite aggressive pharmacological treatment. Chapter 25 on antigens discusses common indoor aeroallergens and measures to control them. Management of hypersensitivity diseases often includes temporarily removing individuals from the implicated environment while abatement efforts are underway or to assess symptom improvement [see 25.2.3.3]. Medical follow-up to assess response to treatment is important prior to building reoccupancy. The risks of building reoccupancy and disease reactivation may preclude an individual's return to an implicated building if assurance of adequate antigen abatement is difficult. This is especially true in HP cases where low-level exposure may cause disease progression in the absence of acute symptoms (i.e., damage may continue even though the patient does not notice it).

8.2.2 Inhalation Fevers

Inhalation exposure to microbially contaminated humidifiers, moldy grain dust during silo unloading, and aerosols containing legionella bacteria have been associated with similar, febrile, flu-like illnesses known collectively as inhalation fevers. Inhalation fevers are self-limited, and respiratory impairment is unusual. The syndromes are notable for very high attack rates among those heavily exposed, even if the persons previously were naive (unexposed) to the inhalant. The incubation period is typically short (e.g., a few hours to less than three days following exposure). Treatment is supportive, and antibiotics have no role because, even if microorganisms are involved, they are not acting as infectious agents. Exposure to the organic dusts seen in agricultural and related settings is uncommon in nonmanufacturing work environments. However, humidifier and Pontiac fevers may be seen in office, residential, and recreational settings.

8.2.2.1 Humidifier Fever Humidifier fever is an influenza-like illness with constitutional symptoms of fever, chills, headache, malaise, and, less prominently, respiratory symptoms [see also 3.4.1]. The syndrome occurs 4 to 12 hours after exposure to aerosols generated from air-conditioning or humidification systems. Humidifier fever may be associated with return to work after time away from a contaminated environment (so-called "Monday miseries"), and symptoms may improve over the work week. The specific pathogenic factors leading to humidifier fever remain to be identified, but the excessive growth of microorganisms within humidification systems seems to be common to all outbreaks. Implicated organisms include amebae (e.g., *Naegleria gruberi*), Gram-positive bacteria (GPB) (e.g., actinomycetes), Gram-negative bacteria (GNB) (e.g., *Pseudomonas* spp.), and bacterial endotoxins.

8.2.2.2 Pontiac Fever Airborne transmission of *Legionella* spp. from environmental sources can cause two forms of legionellosis, that is, Legionnaires' disease [see 8.2.3.1] and the nonpneumonic, flu-like illness known as Pontiac fever. An inhalation fever should be suspected when workers describe a flu-like illness without respira-

tory symptoms following exposure to aerosolized water. Several outbreaks of Pontiac fever have been identified retrospectively, including the one that gave the disease its name (see also 3.4.2). The first recognized outbreak occurred in a county health department building in Pontiac, Michigan, in July, 1968. This outbreak later was traced to the airborne spread of *Legionella pneumophila*, serogroup 1, from a contaminated air-conditioning system (Glick et al., 1978). Seroconversion to this legionella strain was shown in symptomatic persons but not in controls. Diagnostic confirmation of Pontiac fever rests on the finding of typical symptoms, symptom onset after a short incubation period, short illness duration, absence of pneumonia, and demonstration of seroconversion.

8.2.3 Infections

Chapter 9 describes the transmission and control of airborne infectious diseases. Here issues related to infectious disease surveillance and treatment are discussed. Fungal infections are not discussed because these are rare relative to bacterial and viral infections and are unlikely to be seen in normal hosts as a consequence of occupying a building in which fungi are growing. Section 19.2.1 and other references (Rose, 1994) describe work-related fungal infections. Likewise, amebic infections are unusual and are covered in Section 20.2.

8.2.3.1 Legionnaires' Disease Outbreaks of Legionnaires' disease (also called Legionnaires' pneumonia) are relatively uncommon, and most cases are believed to occur sporadically (WHO, 1990; Stout and Yu, 1997). However, Legionnaires' disease may be the most common airborne bacterial infection associated with environmental contamination of office or commercial buildings and one associated with mortality as well as morbidity. The incubation period for legionella pneumonia averages 5 to 6 days from exposure. One to five percent of exposed persons develop illness, and the case-fatality rate may exceed 15%. A person's risk of acquiring Legionnaires' disease following exposure to contaminated water depends on the type and intensity of exposure and the person's health status.

Diagnosis of Legionnaires' disease is based on clinical illness, pneumonia confirmed by chest radiograph, and laboratory evidence of recent legionella infection. A diagnosis of Legionnaires' disease may be confirmed by a positive finding on one or more of the following tests: (a) isolation of the organism from a clinical specimen; (b) demonstration of a four-fold or greater rise in serum antibody titer; (c) demonstration of the organism in a clinical specimen by direct fluorescent antibody stain; or (d) detection of antigen in urine (available only for *L. pneumophila*, serogroup 1) (CDC, 1997a). Legionnaires' disease responds well to specific antibiotic treatment (e.g., erythromycin).

Legionella bacteria are relatively common in natural and man-made water systems. Whirlpools, leisure pools, evaporative condensers, grocery store misters, and cooling towers have been implicated as sources in legionellosis outbreaks (Spitalny et al., 1984; Friedman et al., 1987; Fenstersheib et al., 1990). Besides inhalation of aerosols containing the bacteria, ingestion or aspiration of water containing high concentrations of legionellae has been linked with nosocomial transmission of Legionnaires' disease. Isolation of a *Legionella* sp. from an infected person (a clinical isolate) may facilitate identification of the probable source of work-related infection. Legionellae may be found in more than one water system in a facility, but evidence of causality requires that an environmental isolate match one from a patient (ASTM, 1996).

8.2.3.2 Tuberculosis Tuberculosis (TB) is a contagious disease most often transmitted from person to person by droplet nuclei containing *Mycobacterium tuberculosis*. TB outbreaks have been documented in many indoor environments including health-care settings, prisons, shelters for the homeless, and following naval and air travel (Houk, 1980; CDC, 1989, 1995; Nardell et al., 1991). Transmission is most likely to occur from persons with unrecognized lung or laryngeal disease who are not on adequate therapy and have not been placed in TB isolation. Because individuals with multi-drug resistant TB (MDR-TB) may remain infectious for long periods, outbreaks of MDR-TB have heightened concern about the risk of nosocomial or occupational transmission. TB incidence increased in the U.S. in the 1980s and 1990s, in part related to the high risk for TB among immunosuppressed persons, particularly those infected with the human immunodeficiency virus (HIV).

Active TB should be treated with a combination of medications following the most recent treatment recommendations from the Centers for Disease Control and Prevention (CDC) (CDC, 1994a; www.cdc.gov). Prophylactic therapy should be considered in individuals with demonstrated tuberculin skin test (TST) conversion—a positive test after a history of negative tests—who do not have evidence of active disease. Employees with active pulmonary TB should be restricted from work until they are shown to be noninfectious. Co-workers and other close contacts of employees with active TB should be screened for TST conversion if there was a likelihood of exposure to aerosolized *M. tuberculosis*.

Typically, surveillance for TB is conducted by periodically skin testing high-risk employee groups (e.g., workers in direct contact with patients, clients, animals, or tissues known to have a high risk of infectivity). However, TSTs are neither sensitive nor specific when applied to low-risk populations (e.g., all persons in a building in which a person with active TB worked rather than just those likely to have had close or prolonged contact with the person). Unfortunately, defining contacts too loosely

is a frequent response to administrative pressure to do something following the diagnosis of an active TB case in a work population. Such overtesting may result in unnecessary anxiety and needless preventive treatment. False-positive TST reactions can occur and positive reactions may be due to previous infection or vaccination rather than recent exposure to the sentinel case under investigation. Both prior infection and vaccination are common among workers from countries with a high TB prevalence (e.g., Mexico, southeast Asia, and Africa). OSHA and the CDC can provide information on current recommendations for TB prevention in high-risk and other settings (www.osha.gov; www.cdc.gov).

8.2.3.3 Viral Infections Airborne viral illnesses (e.g., influenza, common colds, measles, rubella, and varicella) are important causes of morbidity in the general population. The transmission of these infections may be increased where large numbers of persons live and work in close quarters in buildings with low outdoor air ventilation rates (e.g., military housingand jails) (Brundage et al., 1988; Richards et al., 1993; Hoge et al., 1994; Tappero et al., 1995). Often, acute viral infections may be considered community-acquired, rather than work-related, even when apparently contracted in office buildings or institutional settings, because the prevalence of these infections is so widespread. Antibiotic treatment is seldom indicated for these viral infections, although ill persons should be excused from work to limit transmission to others. Immunization is the primary means of protecting people from many common viral infections. In the military, vaccination against influenza and common adenoviruses has helped to prevent outbreaks. Increased ventilation may reduce the risk of airborne infections, but there are few studies supporting this assumption [see 10.5.2.1 and 9.8.6.3].

8.2.3.4 Immunization At a minimum, all adults should have current immunizations for the most common communicable diseases (CDC, 1991, 1994b; www.cdc.gov). The airborne or droplet-borne infections for which safe and effective vaccines are available are diphtheria, measles, mumps, pneumococcal diseases, rubella, and varicella. Routine influenza immunization has long been recommended for (a) workers at increased risk for exposure, (b) persons likely to experience complications from infection (e.g., adults with chronic pulmonary or cardiovascular disease and workers ≥65 years of age), and (c) persons who have contact with the latter (CDC, 1996a). However, immunization against influenza is not routine for all occupants of densely populated buildings with recirculated air.

8.3 Approach to Patients With Building-Related Symptoms (BRS)

Mucous membrane irritation of the eyes, nose, and throat, fatigue, and headache associated with building occupan but no identified specific cause are considered BRSs [s 3.2]. Clinical evaluation can be helpful in IEQ investig tions to rule out allergic, toxic, irritant, or infectious di eases because many BRSs and symptoms of BRIs overla and the two syndromes may occur in thesame buildin Hodgson (1989) recommended an approach similar to th following to evaluate patients with BRSs: (a) identify th organ system with the most severe complaints (e.g., th eyes, nervous system, upper respiratory tract, or skir (b) examine workers for physiological abnormalities, (c) physiological abnormality is present, collect occupation and environmental histories to identify potential causes an conduct diagnostic tests, as required, (d) if no physiolog cal abnormality is detected, recommend an engineerir review of the building by a group knowledgeable in po tential causes of BRSs, and (e) correct problems identifie during the building evaluation.

8.3.1 Physiological Tests

A few investigators have identified physiological tests th correlate specific BRSs with measurable abnormalitie (Franck, 1986; Morgan and Camp, 1986; Bascom, 1991; Kjaergaard et a 1992; Koren et al., 1992). Research has provided insight int the potential mechanisms of some BRS complaints, but th tests used in these studies are not routinely available i clinical settings. Franck (1986) associated eye complaint (e.g., dryness, irritation, and inability to wear contact lense with decreased stability of the precorneal tear film, epithe lial damage, and absence of foam in the eye canthus. Oth ers have reported similar findings of corneal film breaku and inflammatory cells in tear fluid. Measurement of th sensory irritation threshold of the eyes to carbon dioxid may be a sensitive method for evaluating eye irritation com plaints related to airborne pollutants (Kjaergaard et al., 1992).

Changes in nasal airflow impedance (i.e., obstruc tion to air flow) have been found in subjects exposed t vapors from carbonless copy paper, with increases i impedance at exposures below concentrations necessar to cause subjective irritation or congestion (Morgan an Camp, 1986). Nasal challenge, with analysis of cells an mediators in nasal lavage, may be useful to study re sponses to irritants in indoor air (Bascom, 1991). In on study, 14 subjects were exposed to clean air and low con centrations of a VOC mixture, followed by nasal lavag (Koren et al., 1992). After VOC exposure, but not clean-ai challenge, there was a statistically significant increase i neutrophils, both immediately and 18 hours later. This finding suggested a physiological basis for the symptom of nasal congestion and irritation that some occupants o problem buildings report. Section 26.3 discusses othe health effects associated with VOC exposures and pos sible effects of MVOCs.

8.3.2 Management of Building-Related Symptoms

Long-term management of BRSs is frequently difficult. Medical treatment with pharmacological agents has not proven useful, other than on a symptomatic basis. Where warranted, reassurance by the treating physician that the annoyance complaints are unlikely to persist or lead to more serious illness is usually helpful to a patient. An employer may be willing to reassign a symptomatic worker to another location or job even in the absence of objective adverse effects, with the expectation that a happy employee and a supportive environment will result in greater productivity and less risk of a crisis situation. Unfortunately, many symptomatic workers without measurable physiological dysfunction are left on their own to develop management or avoidance strategies that enable them to continue working in a problem environment. A preferable approach is environmental assessment [see Chapter 4] and, if necessary, epidemiological evaluation of the potential problem building to identify strategies for remediation. Medical follow-up of affected individuals should take place once control strategies have been implemented.

8.4 Approach to Immunocompromised Building Occupants and Remediation Workers

Immunocompromised persons are at greater than normal risk for illness from exposure to opportunistic pathogens and may require special consideration. Individuals with hematological and other malignancies, HIV infection, diabetes mellitus, renal dysfunction, splenic disorders, alcoholism, cirrhosis, transplanted organs, and those receiving immunosuppressive medications (e.g., high-dose corticosteroids or chemotherapy) have variable degrees of immune system dysfunction (Macher and Rosenberg, 1999). Opportunistic fungal pathogens (e.g., *Aspergillus* spp. and other fungi able to grow at body temperatures) can cause invasive disease in immunocompromised hosts. These agents are common on plant and soil substrates and in outdoor air and are also present in most indoor environments (Arnow et al., 1991; Rose, 1994; Dixon et al., 1996; CDC, 1997b). Therefore, it is difficult to completely prevent exposure to opportunistic fungal pathogens.

Immunosuppressed workers at increased risk of infection and those for whom infection may have especially severe outcomes (e.g., pregnant workers) should be advised by their physicians about what types of activities to avoid and educated in techniques to limit their exposure to infectious agents in the workplace. For example, immunocompromised workers should avoid activities that may expose them to sprays of contaminated water (e.g., work near water-cooled heat-transfer equipment that generates water aerosols) or to airborne fungal or bacterial spores (e.g., decontamination and removal of indoor microbial growth or animal droppings) (CDC, 1997c). These workers should know how to properly use PPE where indicated to limit bioaerosol exposure (Frazier et al., 1994). If potential exposure cannot be eliminated (e.g., TB exposure for a prison or shelter worker), consideration should be given to risk avoidance by temporary or permanent alternative job placement. Immunization may also be advised, although certain vaccines (especially live virus vaccines) may be contraindicated for immunocompromised persons (e.g., measles, mumps, rubella, varicella, and live polio and rabies vaccines) (Frazier et al., 1994; CDC, 1996b).

8.5 Sentinel Health Event Followback of a BRI

The clinical diagnosis of a bioaerosol-related hypersensitivity illness or certain infectious diseases in an individual worker constitutes a sentinel health event and should trigger consideration of an environmental investigation (Rutstein et al., 1983). The recognition of disease in one person may suggest that others who share the same work, residential, or recreational environment may also be at risk (Reingold, 1998). In this circumstance, appropriate intervention extends beyond the individual patient to encompass the at-risk environment and its occupants, with the goals of identifying other cases of disease, providing them appropriate treatment, and eliminating shared hazards. Obtaining a careful occupational and environmental exposure history (Table 8.1) from an affected individual can help direct a subsequent environmental investigation and is part of the hypothesis testing process discussed in Chapter 2.

It is essential to approach an investigation with a team of experienced experts (e.g., physicians, epidemiologists, toxicologists, engineers, IHs, EHPs, and IEQ consultants). Building managers, maintenance personnel, and building occupants themselves should be involved in investigations to ensure that risk factors are carefully sought and accurately identified. An initial inspection of an implicated building is often an early step, during which the investigators look for potential bioaerosol sources and assess the need to collect air or source samples to identify the presence of unusual concentrations or kinds of biological agents [see Chapter 4].

8.5.1 Environmental Monitoring

The decision to proceed with quantitative bioaerosol sampling is guided by the results of the initial worksite survey and the type of illness identified. Bioaerosol sampling to identify relevant exposures in a case of hypersensitivity lung disease may be helpful if positive, that is, if antigens to which a person is known to be sensitive are identified at elevated concentrations. However, while positive results may indicate exposure to potentially antigenic bioaerosols, evidence of exposure does not necessarily identify which of the agents that are present is the one responsible for a worker's hypersensitivity dis-